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Of nature trusts the Mind that builds for aye"—WORDSWORTH

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EDUCATING THE WHOLE MAN

IN his address as 'special visitor' at the commemoration day ceremonies of the Imperial College of Science and Technology, Sir Cyril Hinshelwood once again affirmed his disbelief in the 'two cultures'. He thinks instead that there are simply two attitudes, and that it is the business of universities to encourage the right attitude. Addressing more particularly the younger part of his audience, he proceeded to outline constructively what he meant by the right attitude and how it could be developed.

It is interesting to set Sir Cyril's address beside Sir Mark Oliphant's Frank Tate Memorial Lecture on science and ethics recently delivered at the University of Melbourne and published in the quarterly *Overland*. Sir Mark began by referring to Sir Charles Snow's Rede Lecture, and while he himself is disposed to admit the existence of a gulf between two cultures, he was no less forthright than Sir Cyril in condemning the educational methods that are responsible for perpetuating such a gulf between the scientific disciplines and the humanities. In Australia, there are, Sir Mark said, continual and confused complaints of falling standards in the teaching of science in schools, due to the difficulty in obtaining the services of good science masters ; and the catastrophic result, he said, of the determination of Asian nations to become technologically industrialized in the minimum of time is apparent to all who have examined the schools and universities in those countries. The educational processes through which men pass, from the kindergarten to the university, deliberately preserve the schism in Western culture, and it often seemed to Sir Mark that there is a conspiracy among educationists to create and widen a gap between the two aspects of our culture.

Sir Mark Oliphant thinks that, unfortunately, those who strive most assiduously to make this chasm unbridgeable are the humanists, who continue to fight a rearguard action against science instead of endeavouring to change it into a full and satisfying part of cultural education, such as Sir Cyril Hinshelwood and Sir Eric Ashby, for example, have claimed that it can be. Scientists, on the other hand, Sir Mark believes, are largely either indifferent towards the humanities or are openly contemptuous of what they regard as the imprecision, the paucity of new ideas, and the accumulation and regurgitation of information. In consequence, for example, the philosopher may ignore the great wealth of precisely recorded experience which is science, while those in the faculties of science, technology and medicine are denied the questioning of the basis of their system of natural knowledge which a study of philosophy can contribute.

Sir Mark recognizes the grave threats to the continued existence of mankind which have come from scientific and technological advance ; but though he points out that most of our beliefs and the attitudes which arise from them will not stand up to careful or inspired analysis, and that a completely rational world is neither a possibility nor a desirable state of affairs, he does not, like Prof. C. A. Coulson in his Beckley Lecture, pursue this to its conclusion. Nevertheless, like Prof. Coulson, he recognizes that only through education can we hope to convey to every man some understanding of science and the way it advances, so that he has some idea of the forces which shape his life and his destiny ; and, again like Prof. Coulson, he comes down emphatically in support of making science as much a part of universal education as history or English, so that pupils accept the necessity of knowing something of the structure of the universe as readily as they accept the need for some knowledge of the geography of the world. With proper understanding of this greatest single problem in teaching and the boldness to tackle it experimentally, in whatever ways seem promising, Sir Mark believes the chasm between our two cultures could disappear in a single generation.

Here accordingly, though he began by seeking a rapid bridge of the gap, Sir Mark's thought approaches that of Sir Cyril Hinshelwood, and he believes, too, that science can, and should, be taught in such a way that even those who follow primarily totally different courses have enough knowledge to be sensible about, and sensitive to, the needs of the rapidly changing world in which they live. He does not, however, believe that education about science and the scientific point of view for the ordinary man and woman can be the same as education in science for the budding man of science, but that a totally different approach is required ; and though he makes some important suggestions, on the whole his analysis is scarcely as penetrating as that of Sir Cyril Hinshelwood or Prof. Coulson. If science is to be taught as a general cultural subject in primary and secondary schools, knowledge of Nature, he urges, must be presented as a unity, and it is essential to preserve to the full the questioning, eager approach to the world which is natural in children, making it the very bedrock of the inquiry into Nature which a course in science should be. Every statement of fact should be justified by the evidence, and the real significance of the 'laws' of science, as reasonable approximations, to be discarded as better concepts are born, must be woven into all discussion of them.

Sir Mark points out, too, how science, taught in this way, displaying the contributions which all nations have made to the advancement of science, can help to dispel some of the illusions which feed nationalism : the idea that no knowledge can be secret, but that it only becomes knowledge when shared with and tested by others, is a lesson to be learnt as early as possible. Attacking the illusion that science is a second-rate discipline, he observes very pertinently that the number of scientists is now great enough for them to form a general cross-section of the community, and that among them accordingly will be found the same fraction with the ability to lead, in industry or in government, as among those whose specialized knowledge is in law, history, the classics, economics or commerce. Perhaps a larger proportion of scientists is seriously concerned for the present well-being and the future of mankind, because they appreciate more clearly the realities of nuclear warfare, the nature of the population explosion which threatens our standards of living, and the subtle dangers of man's continuing interference with Nature.

It is essential that man should face up to all the grave responsibilities involved now that the biological future is in his hands, with the increasing effects of the extremely rapid development of ideas and knowledge on the evolution of his social systems, his problems of education and his religion. Sir Mark agrees with Anatol Rapoport that there exist in science ethical principles which are inherent in its pursuit and that there is something universally satisfying about the scientific point of view, particularly as it affects man's relation to his environment. The keystone of science is the pursuit of truth, and Sir Mark maintains in conclusion that the pursuit of knowledge is in no way incompatible with the preservation of human dignity, of the eternal values of art and literature, or with the ethical principles which must govern men's lives. Indeed, science has positive contributions to make to all human activities, and can uphold the basis of value judgments at a time when the religious authority for these is disintegrating.

That last proposition may be debatable, but nevertheless in his conclusion Sir Mark comes very close to both Sir Cyril Hinshelwood and Prof. Coulson, particularly when he pleads for re-thinking the whole basis of education, so that science and its relations with man may permeate the whole fabric of teaching and thus, as sufficient understanding of science and knowledge of its content becomes a natural part of the preparation of every man and woman for life, repair the rift existing between science and other forms of human culture. In his address at the Imperial College of Science and Technology, however, Sir Cyril Hinshelwood laid much less stress on formal teaching, though he agreed that there might be an optimum arrangement of the formal syllabus. The really essential thing is that what is studied at the university should become part of life and should be handled in such a way as to achieve this. The formal teaching, he said, is only a small part of what a university provides, and if the tone and atmosphere of a university are right, general reading, discussion, conversation and argument provide a good deal of the breadth which, as well as depth, is desirable in the educated man. In developing his argument that besides depth, or intensity of experience and technical proficiency, the educated man should seek first of all a broad vision of how his own subject fits into the scheme of things—and that the varied ways in which the painter, the historian, the biologist or the physicist looks at the world, all represent attempts to come to grips with the reality about us—Sir Cyril, by implication, refuted much that Dr. A. P. Rowe advocated about practices and changes in British universities in a recent broadcast talk, "Dry Rot and Redbrick", and which he has also expanded in an article, "The Problem of Freedom and Efficiency in Universities" in *Science and Freedom* for October. Sir Cyril was concerned quite clearly with the average student, not just with the *élite*, and with the teaching which should be given them; and though there is substance in the charges that there is too little scholarship, much trivial research and much waste of public money, Sir Cyril was not concerned directly with these questions. He was concerned that the student should receive a worthy education, and his conception of university teaching is the antithesis of Dr. Rowe's rejection of any idea of a broad education and his suggestion that research should be relegated to research institutes.

Sir Cyril Hinshelwood's reasoned exposition of his conception of the humane or liberal approach deserves careful study. He is convinced that while subjects will wax and wane in importance, and methods be introduced and superseded, there will always be the need to find some satisfaction and understanding in a broad view of the world, and in acquiring the expert skill by which somewhere the student can in due course make an effective contribution. Moreover, it is because he sees the relation of student and teacher in a university as one of partnership, of a guide, rather than oracle, that he maintains teaching and research must remain closely interlinked. In research new knowledge comes into being, and academic studies become part of real life. Work in the laboratory, he observed, with its hopes and disappointments, its difficult decisions when to continue and when to cut losses, its subtle dependence on human relations, is an education in life which the arts scarcely parallel. Finally, he rejected explicitly the idea that research should be concentrated in central institutes devoted solely to that end as likely to imperil the creative attitude, the sense of responsibility and involvement which are developed in the right university atmosphere.

Developing this theme, Sir Cyril pointed out that what we study, whether literature or science, is not part of life and is therefore not humane. How we study is part of life and therefore is humane, and he argued further that the right attitude to all affairs is fostered by the practice of the right approach to our subjects of study. The strenuous effort to see a subject in its true and wide perspective cultivates sanity of judgment in much broader fields. Independent thought, critical assessment of supposed authorities, and sharing of responsibility with our teachers for the views we adopt, engender a healthy mistrust of political pundits and foster the spirit of resistance to the endless petty tyrannies which beset modern life. In words which are echoed in the lectures by Sir Mark Oliphant and Prof. Coulson, Sir Cyril suggests that the need in science to reach one's ends by exacting and precise techniques, whether mathematical or experimental, is a great corrective of the tendency to airy generalization, which is another weakness of the modern world; also that the independent, constructive and, in however modest a degree, creative approach to a subject is the best guarantee that those who have passed through a great scientific school will contribute their full share to the direction and inspiration of industry and public life.

While, therefore, Sir Cyril clearly does not accept much of Dr. Rowe's argument about research and teaching, his address is not a defence of either trivial research or inferior teaching. Rather he calls for teaching of the highest quality, as well as for much independent and critical thinking about methods as well as the content of courses. It may well be that some administrative changes will be needed before ideas such as those outlined by Sir Cyril Hinshelwood and Sir Mark Oliphant are acceptable; further, some changes in the relations between the universities and the schools will be necessary. The changes, however, are not of the kind which Dr. Rowe suggests, and will require much more constructive thought before their shape can be determined. This is one further subject to which attention will no doubt be given when the promised inquiry into higher education is launched; meanwhile, Sir Cyril Hinshelwood and Sir Mark Oliphant have indicated some lines for further thinking and inquiry as well as for profitable experiment.

PAULING'S CHEMICAL BOND

The Nature of the Chemical Bond and the Structure of Molecules and Crystals

An Introduction to Modern Structural Chemistry. By Prof. Linus Pauling. Third edition. (The George Fisher Baker Non-resident Lectureship in Chemistry at Cornell University.) Pp. xx+644. (Ithaca, New York: Cornell University Press; London: Oxford University Press, 1960.) 60*s.* net.

WHEN the first edition of this book was published in 1939 it was immediately recognized as a classic: for many chemists it became a sort of professional bible. I recall clearly my own sense of breathless astonishment at the scale, the self-confidence, the brilliance and certainty of the author's ideas. For the first time the theory of the chemical bond became a unified and homogeneous, sustained theme. Prof. Pauling rightly received his Nobel Prize for being so largely responsible for this.

Now the third edition is in our hands. It is larger, though many of the older pages survive with little alteration. Inevitably some changes were needed in such matters as bond-energies and bond-lengths, to allow for better experimental information now available. But the author has added new material, notably on his order-length relation for resonating bonds, on his theory of metals (here he has carried almost alone the weight of battle against the physicists), and on the new and interesting electron-deficient compounds (of which the bridged molecule of diborane is the simplest example). We should be grateful for all this, particularly in the discussion of metallic bonding and the metallic radii of atoms. Some of us may even be tempted to smile quietly at the new pages devoted to the defence of resonance as being no more 'idealistic' than any other theory of chemical bonding. This is a barbed shaft, aimed in a particular direction; and it hits its mark.

There is no doubt but that this new edition will carry the influence of Linus Pauling a stage further. Yet it is impossible—after twenty-two years use of the earlier edition—not to recognize that the book's very success is a pointer to at least one present weakness. For in order to achieve so wonderful a unification of chemistry, Prof. Pauling was almost forced to adopt just one line of description. This is the description first introduced by Heitler and London in 1927, and extended (very largely) by Pauling himself. We call it the familiar valence-bond, or electron-pair, theory. But what he has not done is to provide a really good critique of this theory, or any sufficiently generous reference to the theory's chief rival, the molecular-orbital theory of Hund, Mulliken and Lennard-Jones.

Let me illustrate both of these points. In the first place, very few people who read the pages here devoted to the relation between the dipole moment of a diatomic molecule and the description of its electronic structure in terms of covalent-ionic resonance will gain any idea of the extreme delicacy which attends any calculation or interpretation of molecular dipole moments. An example of the second criticism occurs in the discussion of the new molecular 'sandwich' molecules such as ferrocene. If the valence bond method is adopted, there are no less than 560 structures to be considered. In nickelocene there are 4,100. Pauling starts his account of this latter compound: "With the assumption that the 4,100 structures have equal weight. . .". Some reference really ought to have been made to the fact that in systems like this, and in a large part of the chemistry of inorganic complexes, the molecular-orbital description is vastly simpler, devoid of sweeping assumptions and easier to visualize. There will be many others as well as myself who regret that ligand field theory is dismissed in just over one page.

Yet these limitations in the book are almost inevitable if the author's theme is to remain as grand and monolithic as the structure of the book demands. If the reader can be kept occasionally reminded that there are alternative ways of thinking about the nature of the chemical bond, he can turn to this new "Nature of the Chemical Bond" and experience all the exhilaration and thrill that come from being in the presence of a master of the art of exposition, a man with an immense knowledge of chemistry and passion for it; and whose influence will remain for so long as wave-mechanical ideas continue to provide our best tool for getting to the heart of the structure of molecules and crystals. C. A. COULSON

LEUKÆMIA

Leukæmia

Research and Clinical Practice. By F. G. J. Hayhoe. Pp. viii+335+28 plates. (London: J. and A. Churchill, Ltd., 1960.) 80*s.* net.

DR. HAYHOE is to be congratulated. His review of one of the widest and most varied fields of practical and experimental medicine is not only remarkably comprehensive but also readable. Each chapter covers a logically delineated region. Difficult questions are not avoided, but a reader fairly new to the subject will not feel choked by incompatible observations or rival theories. Even when discussing subjects to which he has made important original contributions, such as the cytochemistry of the leukæmic cell, Dr. Hayhoe has maintained a critical detachment. The chapter on this subject is one of the most valuable in the book.

There are many other excellent chapters. Outstanding are the critical reviews (Chapter 3) of existing statistical evidence for an increase in incidence of leukæmia in recent years, and for differences in incidence between different countries and races, and the clear account (Chapter 7) of the confusing subject of antigenicity and immunology of the leukæmic cell.

Dr. Hayhoe has surveyed the literature issued up to about the end of 1958. In some fields advances since then have been far-reaching, and I think he would probably modify one or two of his statements to-day. For example, he regards the question whether those conditions variously known as 'homologous', 'runt', and 'wasting' disease are due to a graft-antihost or a host-antigraft reaction as still open (pp. 52-54, and 153). Recent evidence (summarized, for example, in an article by Kaplan and Smithers[1]) leaves little doubt that they are caused by the former.

The statement on p. 61 that "In all this work on leukæmia transmission by filterable agents the type of leukæmia involved has been lymphocytic" is based, I think, on a misconception of the nature of the tumour-filtrate-induced leukæmia of Graffi[2]. Dr. Hayhoe refers to it (p. 60) as lymphocytic, but Graffi and his colleagues describe it as a chloroleukæmia of myeloid type, and others who have examined his material agree. The other possible exception to Dr. Hayhoe's rule, the condition described by Friend[3], is a doubtful case: it is now considered to be of mixed origin[4].

An interesting recent development in this field is reported by Gross[5]. He has observed the induction of myeloid leukæmia, by a filterable agent which usually produces lymphoid leukæmia, in mice protected against the latter disease by thymectomy. It is now clear that leukæmias of myeloid, lymphatic, and perhaps other types may all be transmissible by cell-free agents.

The question of the relation between the so-called 'polyoma' agent (which produces both sarcomata and carcinomata in various organs of mice and other animals inoculated soon after birth) and the leukæmogenic agent, with which it is often associated, has also been considerably clarified since the book was written. The former is a typical virus, producing cytopathic effects in tissue culture, and agglutinating red cells, and differs in many ways from the latter, which is so far recognizable with certainty only by its leukæmogenic effects. As might be expected in such a fertile field of research, other important advances have been made since Dr. Hayhoe closed his survey. For example, analysis of the role of the thymus in filterable agent-induced leukæmia[6-8], and the isolation of a filterable leukæmogenic agent from X-ray-induced leukæmia[9,10] promise exciting future developments in our understanding of this dreaded disease. To these and other investigations Dr. Hayhoe has provided an excellent introduction.

The clinical sections of this book are concise and lucid, and his account of the depressing failures of therapy is relieved by painstaking evaluation of those measures which can reduce distress and occasionally prolong life. The beautiful plates, both coloured drawings and black-and-white photomicrographs, are well chosen, and the excellent plan is adopted of introducing the same plates in several groupings to illustrate different aspects of the conditions described.

The book will be found on the shelves of physicians and laboratory workers for many years to come, and one, at least, of the latter hopes that it will help each group to understand what the other is doing.

M. H. SALAMAN

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FORAGE CONSERVATION

The Conservation of Grass and Forage Crops

By Prof. Stephen J. Watson and Dr. Michael J. Nash. Second enlarged edition. Pp. xiv+758. (Edinburgh and London: Oliver and Boyd, Ltd., 1960.) 84*s*. net.

GRASSLAND and other forages grown for animal feeding provide the basis of livestock industries throughout the world and within most farming systems that involve livestock. Under British conditions, grassland herbage as grazed by the ruminant animal provides the cheapest and in many ways the best method of feeding cattle and sheep. However skilful the management of grass, conservation of the summer surplus will always be required to maintain animals during winter. Since 1940 the British farmer has learnt how to extend his annual grazing season, and this has coincided with a greatly increased level of animal production. Thus, although the winter feeding period is now considerably shorter than a quarter century ago, the need for conservation of grass and other forages is as important and as vital as ever it was. Indeed the need for preserving grass when its quality is at maximum was never more vital to our livestock husbandry systems.

It is therefore a most opportune time for the publication of this book by Prof. S. J. Watson and Dr. M. J. Nash. The new volume offers an enlarged and re-written edition of Prof. Watson's earlier edition published in 1939. Since that time the volume of additional work dealing with fodder conservation has been staggering; but this has served to confirm and to clarify many of the underlying principles of conservation which were broadly known two decades ago. In the earlier edition Prof. Watson had made what was perhaps the first widely embracing attempt to review the experimental work of pre-war days; now he and his collaborator have skilfully collated the work on fodder conservation since that time. This second edition is an authoritative compilation of a massive amount of experimental and practical evidence from all over the world. As such it is bound to be accepted as a book of reference and is likely to remain a classic for a very long time to come.

The traditional methods of fodder conservation as hay or silage have notorious defects: the unreliability of hay in most climates and the serious loss of nutrients normal to both hay and silage as conserved to-day. There has been much controversy as to the relative merits of hay and silage; the authors point out that hay and silage are not competitive but are complementary foodstuffs. The staggering feature which every practical farmer as well as technician should note and ponder on is the huge loss of nutrients that takes place annually during the processes of fodder conservation. The authors show that losses of starch equivalent normal in haymaking under reasonably good conditions are on average as high as 42 per cent; this level is only reduced to 35 per cent by barn drying and the use of other modern techniques.

The losses in silage-making are also high; for example, the summary of data from a large number of experiments shows that while losses in dry matter may be no more than one-sixth, the loss in starch equivalent is more than one-fourth while the losses in digestible true protein exceed one-half. These data are from experimental records; one may properly surmise that the losses encountered during silage preservation on the average farm are greatly in excess of the figures quoted from carefully conducted experiments. The most striking feature in most of our conservation practices is the astounding loss of nutrients which appears to occur. This fact surely adds emphasis to the plea made by the authors (in their preface) for a more complete study than hitherto of the fundamental chemical and biological changes which occur during the process of fodder conservation.

The book is well produced and well edited. The subject index is reasonably adequate and there is a most valuable index of authors occupying some fifty pages towards the end of the book. This is a book of reference which should find a place in every library as well as on the private bookshelf of all those interested in feeding farm animals.

William Davies

SPACE SCIENCE

Introduction to Space

By Dr. Lee A. DuBridge. Pp. xii+93. (New York: Columbia University Press; London: Oxford University Press, 1960.) 20*s*. net.

THE George B. Pegram lectureship is awarded annually to an individual who has made outstanding contributions to modern society. The first recipient was Dr. L. A. DuBridge, president of the California Institute of Technology, who gave four lectures on space science, now published as a book.

The first lecture, entitled "Attaining a Space Orbit", consists of a brief history of American rocket development, followed by a discussion of the technological difficulties involved in placing a satellite in orbit. It includes a simple description of the basic parameters which characterize a selected orbit, and this lecture is perhaps the best of the four.

The second lecture discusses the value of satellites and rockets as observing stations, together with the problems encountered in conveying the results of the observations back to the ground. The author remarks on the difficulty of recovering packages from satellites and suggests we should let future generations struggle with the problem, a pessimism confounded by events shortly after the book was sent to press. The lecture contains a rapid survey of phenomena occurring in space which satellite observations should help to clarify, but it is little more than a catalogue, too superficial for the scientist and too technical for the non-scientist.

The last two lectures are devoted to, respectively, the solar system and the universe, each taking about twenty pages. The lecture on the solar system lists some facts about the Moon and the planets (though Saturn is dismissed with the remark that it will bear a closer look) and mentions some of the problems associated with landing on the Moon, Mars and Venus. The lecture on the universe is more interesting, and the author succeeds in compressing a large amount of material into a few pages without lapsing into unintelligibility.

The book is intended to provide a broad picture for the non-scientist of the use of rockets and satellites in adding to our understanding of the physics of space. As such it is successful within the limitations imposed by its length of ninety pages, and it can be recommended to all those prepared to tolerate occasional maltreatment of the English language. The book contains some good photographs of the major planets.

A. Dalgarno

THE REAL MATTER OF NUCLEAR ENERGY

Metallurgy and Fuels

Vol. 2. Edited by H. M. Finniston and J. P. Howe. (Progress in Nuclear Energy, Series 5). Pp. ix+653. (London and New York: Pergamon Press, 1959.) 147*s*. net.

ANY attempt to collect into one source and to review a large part of the flood of publications on nuclear energy must now arouse gratitude, and unless it is very badly done it must be valuable to workers now overwhelmed by the enormous amount of literature on this subject. "Progress in Nuclear Energy" is a series, now well established, of reviews in volumes each covering a particular field. Volume 2 of "Metallurgy and Fuels" surveys in forty-one chapters work done between the first International Conference on the Peaceful Uses of Atomic Energy at Geneva in 1955 and the end of 1957, on basic materials of nuclear energy and on problems in physical metallurgy arising in the field. Materials considered are uranium, thorium, magnesium and its alloys, plutonium, zirconium, beryllium, control materials, and uranium and thorium oxides. Work on several types of fuel-element is reported. Subjects in physical metallurgy include constitutional work on alloys of nuclear fuel metals; transformation kinetics in uranium alloys and effects of irradiation on matter.

The standard of the reviews in this large volume of 631 pages is generally very high, although one or two fall rather far below the others and the value of publishing these may be doubted. They vary very much in length, in the breadth of field covered and in the detail of reporting: it is not clear whether there was editorial policy on this last point, or whether it was left to authors to choose how each would handle his subject. Weber reviews recent progress in the fabrication and application of dispersion fuel-elements in a long (62 pages) and very well-documented paper, the value and importance of which is at once made clear by the list of 82 publications considered. Grainger covers the important work on elements for gas-cooled reactors with authority in a short but very informative and careful chapter. Typical of shorter chapters on very specialized subjects are Corzine's three-page report on the induction melting of thorium and the chapter by Antill, Butler and Barnes on the electrolytic purification of uranium : each of these appears to be based on only one or two publications and also on material not previously published. The wisdom of publishing material for the first time, if indeed this is occurring, would seem to be open to question. Kroll's statement on the production of uranium billets in Belgium from African ore is interesting, but appears without any references to reports containing details of the work he presents so tersely.

Three very useful chapters present in a non-critical way alloy constitutional diagrams of systems containing plutonium, uranium or thorium. Other reviews tend clearly to present the author's opinion on matters not yet settled. This is, perhaps, inevitable and to those who know the authors it may be valuable, but it might be helpful to have editorial guidance as to the policy followed in some cases.

Work in some of the fields reviewed has been continued. The Second International Conference on the Peaceful Uses of Atomic Energy in 1958 led to the publication of an enormous amount of work on nuclear materials which, it is promised, will be reported in another volume in this "Progress in Nuclear Energy" series. It is indeed valuable that the volume under review collects in one book the scattered information available just before so much new material was published as one event.

It is a pity that the price of this volume (£7 7*s.*) is high enough to discourage many workers in the field from buying their own copies. In a collection of quick surveys of the position in parts of the field this might not be very important, but many authoritative parts of this book would be valuable for frequent reference.

It is to be hoped that further volumes in this series on "Metallurgy and Fuels" will appear regularly and that the high standard of the present volume will be maintained.

G. A. Geach

CONGESTION IN TELEPHONE SYSTEMS

Introduction to Congestion Theory in Telephone Systems

By R. Syski. Chapter 2 by N. H. G. Morris. Pp. xvi + 742 + 14 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1960. Published for Automatic Telephone and Electric Company Limited.) 105*s.* net.

Most engineers and many mathematicians remember with respect the book, "Probability and its Engineering Uses", by T. C. Fry, published in 1928. This pioneering text paved the way for a wide appreciation, in the practical world of technology, of the concepts of mathematical probability and its applications. Now, after thirty years, a worthy successor to Fry's book has appeared. It will undoubtedly become the standard reference for telephone engineers who have occasion to study congestion in the treatment of design problems. But it will become more than this. The book by Mr. Syski, while it contains several chapters dealing specifically with telephone matters, is the first really comprehensive account in book form of the theory of queueing. It is a well-integrated text and includes an account of practically all the ideas which have proved capable of mathematical development in this field during recent decades. Thus, it is likely to become something of a standard work on queueing theory for teachers and students in the field of operations research and, more generally, in institutions where the applied mathematics courses include the topics of probability and stochastic processes.

After an introductory chapter, and one discussing the constructional details of telephone systems, there is a chapter of about fifty pages on probability theory. The latter is well written and may be properly understood by mathematicians. But there seems to be some doubt as to whether the telephone engineer will make much of the rather abstract mathematical formulation. Sample spaces and an introduction to operations with sets may be all right. But the introduction of measure and of Borel spaces will probably prove to be difficult for the engineer unless he has pursued mathematics to an unusual degree. There are nice accounts of random variables, distribution functions and conditional probability, and a feature of the exposition is the wealth of illustrative examples. The account of stochastic processes again seems rather 'highbrow' for the engineer.

The next two chapters are concerned with traffic characteristics and loss systems. Included are introductions to renewal processes generally, and the birth and death process. Waiting systems are treated in a chapter of about one hundred pages and both single- and many- channel systems are studied. These include the systems $M/M/r$, $M/D/r$, $M/G/r$, $GI/M/r$ and $GI/G/r$. Congestion in systems with limited availability is studied in the next chapter, and then there is a chapter on link systems. Some of the most interesting results of recent research are discussed in the chapter on special systems. These results relate to the busy periods, various forms of priority and servicing, early departures, finite queues and queues in series, and to special problems such as the clustering effect, intensity fluctuations, and hunting problems. This chapter should be of special interest to the student starting on research in the theory of queueing. The last chapter deals with some problems special to telephone exchanges.

It is interesting to compare the flavour or atmosphere of this book with that of the earlier book by Fry. Both are heavily mathematical. But the remarkable difference is in the degree of abstraction. Syski has certainly demonstrated that what is called 'abstraction' in mathematics has invaded parts of the subject which are of the greatest application. There is no justification for pursuing mathematical abstraction for its own sake. But, on the other hand, this feature of modern mathematics has become an essential part of the tools needed in various applied fields. We cannot avoid it ; and, one corollary appears to be the necessity for training engineers to a higher level of abstraction than traditional courses contemplate.

L. S. Goddard

The Popular Handbook of Rarer British Birds

By P. A. D. Hollom. Pp. xiv+133+40 plates. (London: H. F. and G. Witherby, Ltd., 1960.) 37*s.* 6*d.*

THIS useful book supplements the "Popular Handbook of British Birds" (1952), edited by Mr. P. A. D. Hollom, which excluded species that had been recorded in the British Isles on less than a dozen occasions. Both works are based on the five volumes of the authoritative "Handbook of British Birds" (1938-41) by H. F. Witherby and others, and although much detail is necessarily omitted they include information of more recent date. The latter feature is especially noticeable in this instance, as accounts and illustrations are given of some thirty species which were not included in the original work at all, having been added to the British list in the intervening two decades.

Many of the new birds are vagrants from America, others from Asia or from southern Europe. Some of them represent groups which found no place in earlier books on British birds—frigate-birds, vireos, New World warblers, New World orioles, tanagers and American sparrows. Several of the newcomers have already been reported more than once, and there are further records of others previously on the list, thanks to the activity of our coastal bird-observatories and the expertise of our field workers. Many of the species are thus likely to come to notice again, and a hand-book covering this aspect of British ornithology is very welcome. The information given for each species is in convenient form, covering identification in some detail and with briefer notes on habits, British occurrences and general distribution. The colour plates not available from the original work are by D. M. Reid-Henry and Peter Scott. LANDSBOROUGH THOMSON

Elementary Titrimetric Analysis

By Dr. A. M. G. Macdonald. Pp. viii+133. (London: Butterworths Scientific Publications, 1960.) 12*s.* 6*d.*

THIS book deals exclusively with titrimetric procedures, and although it has been written primarily for students in schools and junior technical colleges, it also deals in an informative manner with the application of redox titrations and the use of complexanes in titrimetry, and these chapters would be well worth the attention of somewhat more advanced students.

The chemical purist might quarrel with Dr. Macdonald's definition of the gram equivalent weight of the acid as the weight which "furnishes one gram of hydrogen ion", and might prefer the more exact definition which would relate to '1·008 gm. of hydrogen ion'. Indeed, the 1957 "Table of Atomic Weights" given at the end of the book quotes atomic weights to three decimal places, and the student might often ponder as to why the gram equivalent weight of sulphuric acid is given as 49 and not as 49·041.

The reviewer also feels that some chapters could have been presented in a clearer manner to the student if diagrammatic illustrations were used; for example, in the chapter on oxidation potentials, a sketch of a redox electrode with salt bridge to a calomel electrode would have been most informative.

These points are, however, minor criticisms, for the book is well balanced and introduces the student gradually to the more difficult exercises of volumetric quantitative analysis. The general descriptions of indicator applications, hydrogen ion concentration, etc., are considered to be completely adequate for the average student, and the theoretical implications have been clearly and concisely described.

In this context the book will prove valuable not only to the student, but also to the teacher of chemistry who has to present the subject of titrimetric analysis to his classes in a manner which makes the theoretical implications easily comprehensible, and the practical exercises suggested are well balanced and cover a wide and useful range of analytical procedures. D. T. LEWIS

Life's Long Journey

By Kenneth Walker. Pp. 191. (London: Victor Gollancz, Ltd., 1960.) 21*s.* net.

THE aim of Dr. Kenneth Walker's latest book is to show that mechanistic views of life are long outdated, and that the universe is essentially spiritual in essence. To arrive at his ultimate conclusion that "it is to religion . . . that man will have to turn for help if he is to evolve any further", Walker roundly assails all neo-Darwinians as well as those who condition our minds by too much science.

The book is unsatisfactory in many respects. Few people will accept Walker's disarming prefatory note that his amateur acqaintance with biology makes him in some way more fitted to interpret its main hypothesis than those who have studied more deeply. His interpretations of Darwin's theory of natural selection do not always represent the views of the progenitor. Dogmatic reiteration of the purposefulness of life does not necessarily make life more purposeful, and is scarcely in keeping with a scientific mode of thinking. The mystical references to Eastern religions as sources of revelation to those to whom the mysteries are vouchsafed are unlikely to console those who profess Western faiths, and who read that "we live in a post-Christian age and an age in which economic man has been allowed to usurp the rights of the whole man". Walker's wish was that this book would help many to look again at the meaning of life; it is unfortunate that its appeal will, for the most, be to those whose minds are already made up. T. H. HAWKINS

The Lichen Flora of the United States

By Bruce Fink. Second printing. Pp. xii+426+47 plates. (Ann Arbor: University of Michigan Press; London: Angus and Robertson, Ltd., 1960.) 12.50 dollars; 50*s.*

THIS is an exact copy of the original edition published in 1935, even to such obvious errors as the transposition of captions on Plate 42. Unfortunately, the erratum slip issued with the former volume has not been reproduced. It seems a pity the opportunity was not taken to rewrite the accounts of such genera as have been revised in North America during the past quarter century, but to do so would doubtless have led to a somewhat uneven treatment of the flora as a whole. Naturalists in the United States will be glad to have this convenient basic summary of their lichen flora once more available. R. W. G. DENNIS

CALENDAR OF CENTENARIES, 1961

JANUARY

4 *Hales, Stephen* (1677–1761), English clergyman who carried out fundamental researches in plant and animal physiology and was awarded the Copley Medal of the Royal Society in 1739.

8 *Clegg, Samuel* (1781–1861), English gas engineer, pioneer in gas lighting.

12 *Baldwin, James Mark* (1861–1934), American philosopher and psychologist.

17 *Hall, Sir James* (1761–1832), Scottish geologist who carried out laboratory experiments to investigate and illustrate geological phenomena.

18 *Goldschmidt, Hans* (1861–1923), German chemist whose name is associated with the thermit process for the reduction of metallic oxides.

22 *Bacon, Francis* (1561–1626), English philosopher whose works include the "New Atlantis" in which is described a model college for the experimental study of Nature.

22 *Tiedemann, Friedrich* (1781–1861), German physiologist and anatomist.

FEBRUARY

15 *Whitehead, Alfred North* (1861–1947), English mathematician and philosopher.

15 *Guillaume, Charles Edouard* (1861–1938), Swiss physicist, awarded the Nobel Prize for Physics in 1920 for his work on nickel–steel alloys.

24 *Santorio, Santorio* (1561–1636), Italian physician and physiologist, author of the medical classic, "Ars de Statica Medicina" (1614).

APRIL

6 *Giesecke, Karl Ludwig* (1761–1833), German mineralogist who travelled extensively in Greenland and located the unique occurrence of cryolite there.

8 *Peurbach, Georg* (1423–61), professor of astronomy in Vienna who revived observational astronomy in western Europe.

MAY

3 *Vallisnieri, Antonio* (1661–1730), Italian physician and naturalist who made valuable observations on fossils and the origin of springs.

13 *Fitton, William Henry* (1780–1861), Irish physician and geologist, author of an important memoir on the strata below the Chalk in south-east England.

14 *Simpson, Thomas* (1710–61), English mathematician, author of a number of mathematical books.

16 *Henslow, John Stevens* (1796–1861), professor first of mineralogy, then of botany, at Cambridge, who nominated Charles Darwin as naturalist for the voyage of the *Beagle*.

JUNE

7 *Rennie, John* (1761–1821), Scottish civil engineer, constructor of canals, bridges and harbours.

18 *Hodgkinson, Eaton* (1789–1861), English civil engineer, noted for his researches on the strength of materials.

24 *Gregory, David* (1661–1708), Scottish mathematician, who, as professor of mathematics in Edinburgh, gave the first public lectures on the Newtonian theory.

29 *Mayo, William James* (1861–1939), American surgeon who, jointly with his father and brother, founded the Mayo Clinic, Rochester, Minnesota.

30 *Hopkins, Sir Frederick Gowland* (1861–1947), English biochemist who received (jointly with C. Eijkman) the Nobel Prize for Medicine and Physiology in 1920.

JULY

8 *Thomson, Sir John Arthur* (1861–1933), Scottish biologist, author of many technical and popular works.

AUGUST

8 *Bateson, William* (1861–1926), English biologist who carried out an extensive investigation of the laws of variation and heredity.

10 *Wright, Sir Almroth Edward* (1861–1947), English bacteriologist and pioneer of vaccine therapy.

SEPTEMBER

8 *Belidor, Bernard Forest de* (1693–1761), French military engineer, author of works on engineering and hydraulics.

17 *Bose, Georg Mathias* (1710–61), German natural philosopher who carried out many experiments with static electricity, some of which he described in a poem, "Die Elektricität" (1744).

19 (or 9) *Musschenbroek, Pieter van* (1692–1761), Dutch physicist who invented a pyrometer and who was concerned with the discovery of the Leyden jar.

24 *Pitiscus, Bartholomaeus* (1561–1613), German mathematician who prepared an early work on trigonometry.

OCTOBER

13 *Cubitt, Sir William* (1785–1861), English civil engineer, responsible for the construction of important docks and canals.

NOVEMBER

7 *Sherrington, Sir Charles Scott* (1861–1952), English physiologist, awarded the Nobel Prize for Medicine and Physiology in 1932 (jointly with E. D. Adrian) for researches on the nervous system.

30 *Dollond, John* (1706–61), English optician, who received the Copley Medal of the Royal Society for his work on achromatic lenses.

30 *Tennant, Smithson* (1761–1815), English chemist, discoverer of osmium and iridium.

DECEMBER

17 *Kennelly, Arthur Edwin* (1861–1939), American physicist who independently predicted the existence of the Heaviside layer.

24 *Pons, Jean Louis* (1761–1831), French astronomer who discovered more than thirty comets.

25 *Gregor, William* (1761–1817), English chemist and mineralogist, discoverer of titanium.

INTERNATIONAL GEOPHYSICAL CALENDAR FOR 1961

THE accompanying International Geophysical Calendar 1961 indicates some special days and intervals for special attention for geophysical experiments and analysis. It serves to encourage world-wide co-ordination of observation or analysis of those geophysical phenomena which vary significantly during the course of a year. These phenomena are mainly in the scientific disciplines dealing with the Earth's atmosphere. In some experiments, such as the routine measuring of variations of the Earth's magnetic field, the observing and analysis programmes at observatories are carried out at a uniform level throughout the year; in these cases the Calendar is not needed. However, in many other experiments (for example, rocket experiments), it is not practical or meaningful to carry out the same programme on each and every day. Here the Calendar can provide a useful mechanism for co-ordination. Experimenters will know that their colleagues in other laboratories and in other disciplines will tend also to carry out experiments on the days or intervals marked on the Calendar. In this way, results of experiments may be more easily and usefully compared.

In some scientific fields, international scientific organizations have made specific recommendations for programmes to be done on days or intervals marked on the Calendar. In others, the arrangements are informal or self-evident. Some examples are given below, along with the criteria for selection of dates.

Copies of the Calendar can be obtained from the Secretary-General, U.R.S.I., 7 Place Emile Danco, Brussels 18.

Regular World Days

These are three consecutive days each month. They always come in the middle or just after the middle of the month, and include where possible the times of equinox and solstice. They come in the middle of the week—Tuesday, Wednesday and Thursday. The groups of Regular World Days are evenly spaced through the year so far as it is practical. They include wherever possible days of solar eclipse and meteor showers. One Regular World Day each month (always Wednesday) is designated the Regular World Day of highest priority.

The Regular World Days are intended for experiments or observational programmes or analysis programmes which as a practical matter can be done only about 10 per cent of the time and should be spaced through the year. Further, it is suggested that whenever there are no special reasons for selecting some other days, the Regular World Days be used for unusual or special experiments. This applies perhaps particularly to scientific fields in atmospheric geophysics, including aspects of cosmic rays, meteorology, airglow, ionosphere, geomagnetism, aeronomy. Examples in ionospheric physics are: oblique incidence pulse transmission and reception; absorption measurements by pulse reflexion technique; extended observing schedule for whistlers and very low-frequency emissions; vertical-sounding observing schedule faster than normal; more detailed reduction of vertical-sounding ionograms by f-plot, h'-plot, etc.; hourly reduction from ionograms of F-region true height parameters hc and qc.

The Regular World Days with highest priority are for work which can be undertaken for only one day each month. All the foregoing examples apply if the rate of three days per month is not necessary or proves to be too heavy. A specific example is the recommended programme for 1959 and onwards of exchange of copies of original ionograms in ionospheric vertical sounding work, made by the International Scientific Radio Union–International Geophysical Year (URSI–AGI) Committee; this recommends that ionograms for the highest priority Regular World Day each month (and also one disturbed period each year) be sent to World Data Centres for interchange.

Regular World Intervals

These are ten consecutive days in each quarter year, selected to include the three Regular World Days of the month and also the times of the equinox or solstice. If possible, they include days of solar eclipses and meteor showers. The selectors have also tried to avoid week-end days and in addition holidays which are widely observed.

The Regular World Intervals are intended for experiments which for practical reasons cannot be carried on continuously, but for which statistics of seasonal variations are especially needed. Ionospheric drift and high-atmosphere wind measurements are two examples. Schedules for interchange of sample detailed data in several disciplines have made use of the Regular World Intervals. In some network observational programmes, both the Regular World Intervals and the Regular World Days are used, in order to get the variations throughout the year but with improved statistics at the equinoxes and solstices.

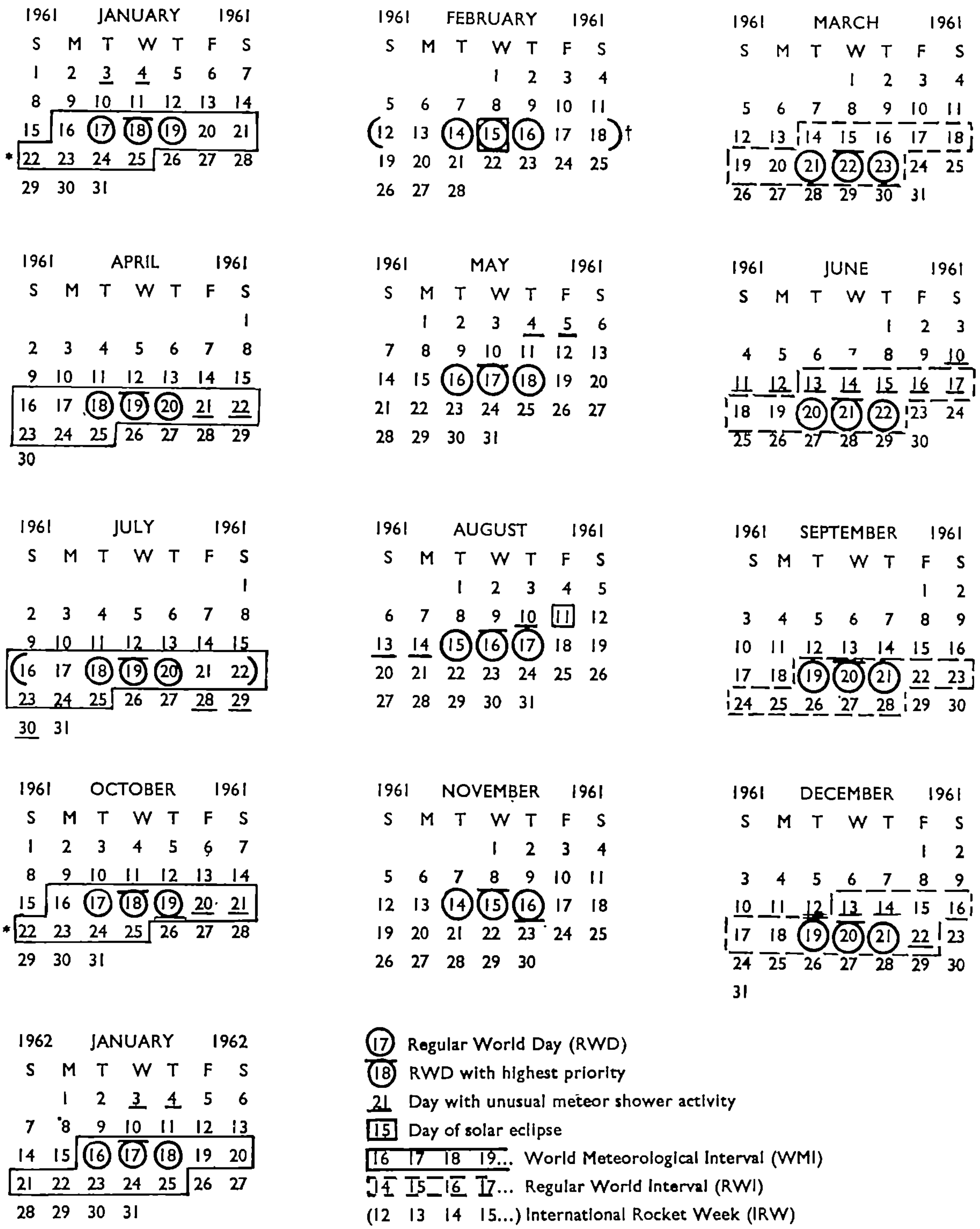
World Meteorological Intervals

The World Meteorological Intervals are ten consecutive days each quarter year, but displaced one month from the equinoxes and solstices. They are intended to cover the times of marked seasonal change in certain meteorological phenomena which tend to come about a month after the equinoxes and solstices. They have been chosen through the World Meteorological Organization and the Committee on Space Research of the International Council of Scientific Unions as the period from the 16th to the 25th of January, April, July and October, with January and October designated by the Committee on Space Research as the more important ones.

The World Meteorological Intervals now are primarily periods for carrying out synoptic meteorological rocket programmes, with stations obtaining atmospheric profiles up to 50 km. or more, at least once daily during the ten-day intervals. These Intervals have also been used during and since the International Geographical Year for balloon sounding programmes either with special instruments or launchings to unusually high balloon altitudes.

International Geophysical Calendar 1961

Issued November 1960 by the International World Day Service under the auspices of the International Scientific Radio Union



1961 JANUARY 1961

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	⑰	⑱	⑲	20	21
*22	23	24	25	26	27	28
29	30	31				

1961 FEBRUARY 1961

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
(12	13	⑭	$\boxed{15}$	⑯	17	18)†
19	20	21	22	23	24	25
26	27	28				

1961 MARCH 1961

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	㉑	㉒	㉓	24	25
26	27	28	29	30	31	

1961 APRIL 1961

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	⑱	⑲	⑳	21	22
23	24	25	26	27	28	29
30						

1961 MAY 1961

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	⑯	⑰	⑱	19	20
21	22	23	24	25	26	27
28	29	30	31			

1961 JUNE 1961

S	M	T	W	T	F	S
				1	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	⑳	㉑	㉒	23	24
25	26	27	28	29	30	

1961 JULY 1961

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
(16	17	⑱	⑲	⑳	21	22)
23	24	25	26	27	28	29
30	31					

1961 AUGUST 1961

S	M	T	W	T	F	S
		1	2	3	4	5
6	7	8	9	10	$\boxed{11}$	12
13	14	⑮	⑯	⑰	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

1961 SEPTEMBER 1961

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	⑲	⑳	㉑	22	23
24	25	26	27	28	29	30

1961 OCTOBER 1961

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	⑰	⑱	⑲	20	21
*22	23	24	25	26	27	28
29	30	31				

1961 NOVEMBER 1961

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	⑭	⑮	⑯	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

1961 DECEMBER 1961

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	⑲	⑳	㉑	22	23
24	25	26	27	28	29	30
31						

1962 JANUARY 1962

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	⑯	⑰	⑱	19	20
21	22	23	24	25	26	27
28	29	30	31			

⑰ Regular World Day (RWD)

⑱ RWD with highest priority

$\underline{21}$ Day with unusual meteor shower activity

$\boxed{15}$ Day of solar eclipse

16 17 18 19... World Meteorological Interval (WMI)

14 15 16 17... Regular World Interval (RWI)

(12 13 14 15...) International Rocket Week (IRW)

Notes : (*) WMI in January and October considered most important

(†) Feb. 15, 1961 : RWD with highest priority

International Rocket Weeks

These have been chosen for 1961 by the Committee on Space Research as (1) February 12–18 and (2) July 16–22. The first includes the time of the total solar eclipse of February 15 and is intended for study of solar effects. The second was selected for study of (northern hemisphere) summer atmospheric structure.

The International Rocket Weeks provide two periods during the year when rocket studies of the atmosphere and of the Sun will be on as nearly a synoptic basis as is possible at this stage of the science and technology. More detailed recommendations by Working Group 2 of the Committee on Space Research (chairman, J. Bartels) appear in the *COSPAR Information Bulletin*.

Other Special Days

The 1961 Calendar marks the days of solar eclipses —February 15 (total) and August 11 (annular). Some special programmes may be expected to be carried out in appropriate parts of the world to study eclipse effects on the Earth's atmosphere. It is especially important that the record of solar activity during and near the times of eclipses be as full as possible. Many observatories interested in solar activity issue specially detailed reports of their observations on eclipse days to assist the interpretation of the geophysical efforts. Ionospheric stations generally increase their observing programmes on eclipse days even if the magnitude of eclipse at their position is small.

Also shown on the 1961 Calendar are days when the activity of meteor showers is unusually high. Geophysicists using meteor techniques often intensify their observing programmes on these days. Attention is also directed to these days in case ionization produced by meteors may account for unusual effects in other geophysical experiments.

Special Intervals not appearing on 1961 Calendar

The International Geophysical Calendar marks only those dates which can be selected a long time in advance, either by general agreement or, in the case of eclipses and meteor showers, by reliable long-term prediction. Periods of great magnetic, auroral and ionospheric disturbance are also of great geophysical interest and world-wide co-ordination of observation is clearly desirable. This is also done under the auspices of the International World Day Service, in close collaboration with the International Scientific Radio Union Central Committee on URSI-grams, in the programme for the immediate designation of 'Geophysical Alerts' and for selection on a current basis of Special World Intervals (SWI). Arrangements for receipt of such information by telegram or radio broadcast can be made, as may be practical, with one of the solar-geophysical Regional Warning Centres, the telegraphic addresses of which are: (Western Hemisphere) AGIWARN WASHINGTON (U.S.A.); (Western Pacific) AGI KOKUBUNJI (Japan); (Eurasia) NIZMIR MOSCOW (U.S.S.R.); (Western Europe) either IONOSPHARE DARMSTADT (GFR) or GENTELABO PARIS (France) or AGI NEDERHORSTDENBERG (Netherlands). The meteorological telecommunications network co-ordinated by the World Meteorological Organization carries such information once daily soon after 1600 U.T. Descriptions of the Geophysical Alerts and Special World Intervals plan can be obtained from these Centres or from the Secretary of the International World Day Service. Many geophysical stations increase their programmes or carry on special experiments during disturbed periods; the Geophysical Alert and Special World Intervals programme serves to co-ordinate this on a world-wide basis and is especially useful for stations not near the auroral zones, where the beginning of a major disturbance may not be immediately apparent from local observations.

The International World Day Service, in close collaboration with the Central Committee on URSI-grams, also fosters arrangements for prompt notification of major solar flare events which have important and sometimes long-lasting geophysical effects. These notifications are also done through the Regional Warning Centres.

Calendar Records

A summary record of significant solar and geophysical events is being prepared as a Calendar Record for the International Geophysical Year period and also for 1959. This work is now under the auspices of the International World Day Service. If these volumes prove to serve a useful purpose, similar Calendar Records may be compiled for 1960 and 1961.

The International World Day Service was established in 1958 by the International Council of Scientific Unions and is administered by the International Scientific Radio Union, 7 Place Emile Danco, Brussels 18, Belgium. Its Steering Committee consists of A. H. Shapley (International Scientific Radio Union), M. Nicolet (International Union of Geodesy and Geophysics), and J. F. Denisse (International Astronomical Union), with R. Coutrez (International Scientific Radio Union) as secretary. It obtains nominal support from the Federation of Astronomical and Geophysical Services of the International Council of Scientific Unions. Mr. Shapley, National Bureau of Standards, Boulder Laboratories, Boulder, Colorado, U.S.A., serves as spokesman for the International World Day Service and its correspondent to other groups of the International Council such as the International Committee of Geophysics and the Committee on Space Research.

The International Geophysical Calendar for 1961 has been drawn up by A. H. Shapley and J. V. Lincoln in consultation with the International Scientific Radio Union, the International Union of Geodesy and Geophysics and the International Astronomical Union, both directly and through the International Committee on Geophysics and the Committee on Space Research. Recommendations also have come from representatives of the World Meteorological Organization and from interested individual scientists. A similar Calendar was issued for 1960 along the lines of the calendars for the International Geophysical Year and the International Geophysical Co-operation 1959 issued under the auspices of the Special Committee of the International Council of Scientific Unions for the International Geophysical Year, and described in the International Geophysical Year Instruction Manual for World Days and Communications, *IGY Annals*, VII (Pergamon Press, 1959).

A. H. SHAPLEY

A NEW METHOD FOR DATING ANCIENT GLASS

By ROBERT H. BRILL and HARRISON P. HOOD

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THE majority of glasses surviving from most historical periods have soda-lime-silica or potash-lime-silica compositions. Glasses with these compositions, similar to some in use to-day, are usually regarded as substances of considerable chemical durability in comparison with many other materials; that is, they are resistant to extensive chemical attack except under unusual or extremely corrosive conditions. Actually, however, even in rather mild chemical environments, glasses of this kind, particularly those having high soda or potash content, are subject to a slow chemical deterioration in the presence of water. The accumulated effect of this slow but persistent hydrolytic attack may be readily seen during a visit to any museum displaying a collection of archæological objects of glass which have been weathered because of burial in soil or exposure to the elements for long periods of time.

Weathering effects on glass appear most often in the form of crusts of decomposition products and show a remarkable variability in appearance resulting from variations in chemical composition and weathering conditions. A number of investigators[1–6], beginning with Brewster in 1863, have made microscope studies of these weathering crusts and have noted that in cross-section the crusts can often be resolved into fine laminar structures. The individual layers of these structures range in thickness from about 0·3μ to perhaps 15μ, and are responsible, through interference phenomena[3], for the striking and colourful iridescence so familiar in archæological specimens of glass.

Most of the work done to date has been largely phenomenological, but it has been suggested that the laminar structures are the result of a mechanism similar to that producing Liesegang rings[6,7]. An alternative explanation, however, may be deduced from the realization that the weathering crusts on archæological objects of glass have been formed over extended periods of time and that the appearance of minute individual layers might be a reflexion of some periodic or cyclical change which was imposed during the long time of exposure. For example, seasonal variations in temperature or alternating periods of rainy and dry seasons might be responsible for the lamination effects. Since such phenomena occur in yearly cycles, the exciting possibility arises of dating archæological specimens of glass with extreme accuracy simply by counting the number of individual layers comprising weathering crusts. This possibility is strengthened by our experiences with the leaching of borosilicate glasses involved in the production of 'Vycor' brand glass. When leaching is carried on under very carefully controlled constant conditions, perfectly clear non-laminar sections an inch in thickness are readily obtained. However, every small change in temperature or concentration of the bath during leaching records the event permanently with a discontinuity of refractive index in the leached specimen.

Table 1

Sample	Date based on archæological evidence	Excavation date	Layers counted	Date calculated for onset of weathering
J-5	Submerged **1781**	1935	150–**156**–170	1765–**1779**–1785
J-4	Buried **1639–1670**	1935	256–**266**–273	1662–**1669**–1679
J-6	Buried *c.* **1660–1675**	1935	261–**271**–286	1649–**1664**–1674
J-2	Submerged *c.* **1600–1640**	1956	295–**310**–320	1636–**1646**–1661
Pl-1	Buried **1676** or later	1959	259–**269**–289	1670–**1690**–1700
PR-5	Submerged **1692**	1959	258–**268**–274	1685–**1691**–1701
SA-2	Submerged **1621**	1959	319–**334**–349	1610–**1625**–1640
N-4	Manufactured eighth–tenth century	*c.* 1950	**930** ± 30	A.D. **1020** ± 30
N-9	ninth–thirteenth century	*c.* 1950	**775** ± 20	A.D. **1175** ± 20
N-11	ninth–thirteenth century	*c.* 1950	**810** ± 25	A.D. **1140** ± 25
S-1*b*	Late third–early seventh century	1960	**1582** ± 10	A.D. **378** ± 10

In order to test this hypothesis of 'seasonal growth' and explore the dating possibilities, samples were taken from a number of glass objects which had been submerged or buried for known time-intervals. These samples were mounted in plastic, polished, and observed in cross-section under a microscope. The number of individual layers within complete weathering crusts was counted. Typical results are shown in Table 1.

The values reported in Table 1 are averages computed from several independent counts on replicate mountings along with estimated maximum and minimum values. By subtracting the number of layers counted from the known dates of excavation, dates were obtained representing the years when weathering started. The agreement of the dates calculated and those known from archæological evidence is indeed sufficient to substantiate that, for these samples at least, the decomposition proceeded according to a seasonal schedule.

It is reasonable, therefore, to assume that within the limitations to be mentioned below, it is possible to determine total times of exposure to weathering conditions for other glass objects by this counting technique. The results in Table 1 present a rather strong case for the applicability of the method to the Colonial glasses studied, and the date determined for the Sardis fragment leads one to believe that it should be useful for dating more ancient glasses as well. The precision of ± 10 years (at an estimated 90 per cent confidence-level) for a number of counts on duplicate mountings of this sample emphasizes the potential accuracy of the technique. The case is not quite so convincing, however, for the Nishapur samples, because they unfortunately cannot be as precisely dated by archæological evidence, and one is, therefore, not certain as to what accuracy has been achieved. There is a need for further work on samples of more ancient origin and such work is being continued at this laboratory.

Although the mechanism of the hydrolytic attack of glass has not been completely elucidated, it almost certainly involves a selective leaching of 'the more soluble ingredients', principally the alkali metal oxides, leaving a hydrated residue of high silica content. On the basis of the results of this investigation, it seems reasonable to postulate that any appreciable and prolonged variation in temperature or availability of water seems to be accompanied by an irreversible change in the structural state of the siliceous residue. This structural change apparently persists even after the original conditions are restored, thus forming a discrete individual layer. Since the layer formed is porous, water may diffuse through from the surroundings during the next 'active' cycle and renew the hydrolytic attack on the body of the remaining glass.

In short, the total extent of weathering must have a complicated dependence on composition, temperature, time and chemical factors such as pH ; but the number of individual fine layers appears to depend only on the number of extended temperature cycles or moist – dry cycles experienced, that is, on the number of years of exposure to the elements.

It is not the case, of course, that every sample of archæological glass can be dated satisfactorily by this method. To begin with, the weathering layer must have been preserved intact, a requirement which unfortunately is not met by the majority of fragments available for study. In addition, the conditions of burial must have contained a yearly cyclical factor such as those mentioned above, and the glass itself must be of a composition which confers relative instability towards hydrolytic attack. Among the many objects examined were pieces with crusts of such fragility that they could not be mounted and polished without damaging the laminar structure. This was especially noticeable in Roman glasses. However, with improved techniques of sample preparation, this method of dating may be extended to many more objects. More refined methods of manipulation should also reduce the errors inherent in the counting procedure, for it is on occasion difficult to distinguish individual layers. This is the major source of experimental difficulty encountered and accounts largely for the ranges of uncertainty included in the reported values.

It should be emphasized that this technique does not yield a date of manufacture or use, but rather a date at which the weathering process apparently started. To be useful as a dating technique, it must be further assumed that the weathering process started at the time of burial or submersion. Among the objects included in Table 1, one instance was found where this condition was not fully met. This sample, *J*–4, a fragment of window glass from Jamestown, showed a curious but not uncommon type of decomposition which did not proceed uniformly from the surface of the fragment, but rather proceeded sporadically from randomly located nuclei on the surface, forming cone-shaped intrusions into the body of the glass. These intrusions or 'plugs' have laminar structures which could be counted, but yielded different dates. It is quite evident that all these plugs did not start growing at the same time, but rather that various induction periods lapsed before growth started at some nuclei. The 'oldest' plug found indicated a starting date of 1669. It is most probable that this date represents a date of burial closely coinciding with the date the building was razed. An alternative interpretation might be that the 1669 date refers to a date when the glass was still incorporated as part of a window, thus being exposed to weathering conditions for some years before burial in the soil. Because of the archæological data, the first interpretation seems preferable, although the range of probable error around the 1669 date would make either position tenable.

Perhaps the most important aspect of this investigation is that this method may provide the archæologist with a simple and exceedingly accurate means for dating archæological sites where suitable fragments of glass are found.

Work will be continued at this laboratory on the refinement of experimental procedures for this method, the extension of the method to other archæological materials and on the extraction of climatological data from the layer patterns. When further data have been collected on additional ancient glass samples, the findings will be published in the *Journal of Glass Studies*.

We thank Mr. Paul N. Perrot and Dr. Axel von Saldern, director and curator of The Corning Museum of Glass, for their assistance and encouragement during the course of this work.

Description of Samples

The samples designated with the letter *J* in Table 1 were supplied by Mr. J. Paul Hudson, museum curator at Colonial National Historical Park, Jamestown, Virginia. His descriptions of the samples follow :

J–5. Base from English wine bottle, 1775–81 period. Found inside the hull of a British ship at the bottom of the York River, off shore from Yorktown, Virginia, in 1935. The ship was sunk during the siege of Yorktown in 1781. *J*–4. Small piece of window glass from the First State House at Jamestown, built about 1639, burned or razed about 1670. The fragment was found in damp soil in 1935. It had probably been buried in the soil about 1670. *J*–6. Neck fragment from an English wine bottle of the 1660–75 period. Unearthed in damp soil near a brick foundation of a Jamestown house, 1650–75 period. It appears that the fragment had been buried in the soil from about 1675 until 1935. *J*–2. Neck fragment from a Dutch gin or spirit bottle of the 1600–40 period. Found in water in a Jamestown well in 1956, in association with artefacts of the 1600–40 period.

Pl–1 is a fragment of a small medicine bottle of the sort in common use during the seventeenth century. It was uncovered during the excavation of the site of the Joseph Howland House at Rocky Nook, Kingston, Massachusetts, in 1959. Mr. Harry Schnabel, supervisor of archæology at Plimoth Plantation in Plymouth, Massachusetts, has associated the west cellar of this building, where the fragment was found, with a period of occupation beginning in 1676. *PR*–5 was taken from the neck of a wine bottle recovered during a dredging operation in 1959 at Port Royal. Mr. C. Bernard Lewis, director of the Institute of Jamaica in Kingston, Jamaica, supplied this bottle, which has been presumed to have been submerged since the earthquake of 1692, when a portion of the city of Port Royal sank below the surface of the sea. *SA*–2, another bottle fragment, was salvaged from a shipwreck off Bermuda by Mr. Mendel Peterson, of the Department of Armed Forces History at the Smithsonian Institution. Mr. Peterson believes that the preponderance of evidence points to this wreck being the remains of the Spanish ship *San Antonio* sunk in 1621, but that a possibility exists that it could be the *Vega* sunk in 1639. *N*–4, *N*–9 and *N*–11 are from the collection of the Corning Museum of Glass. They are Islamic specimens said to have been excavated about 1950 in Nishapur, Iran, at the sites Imam-Zadeh (Ghazi-Abad), Deh-Sheikh and Torb-Abad. The best available estimates for dates of manufacture are those listed in the table. Sample *S*–1 b, is a small lump of glass excavated in 1960 near the House of Bronzes at the site of the ancient city of Sardis in Turkey. Prof. George Hanfmann of the Fogg Museum believes that this building was constructed some time between the late third and early seventh centuries A.D., and that it was destroyed in about A.D. 616.

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[2] Fowler, J., *The Archaeologia*, **46**, 65 (1880).

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IMMUNOLOGICAL TOLERANCE*

By PROF. P. B. MEDAWAR, F.R.S.
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'IMMUNOLOGICAL tolerance' may be described as a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response. The term first came to be used in the context of tissue transplantation immunity, that is, of the form of immunity that usually prohibits the grafting of tissues between individuals of different genetic make-up; and it was used to refer only to a non-reactivity caused by exposing animals to antigenic stimuli before they were old enough to undertake an immunological response. For example, if living cells from a mouse of strain *CBA* are injected into an adult mouse of strain *A*, the *CBA* cells will be destroyed by an immunological process, and the *A*-line mouse that received them will destroy any later graft of the same origin with the speed to be expected of an animal immunologically forearmed. But if the *CBA* cells are injected into a fœtal or new-born *A*-line mouse, they are accepted; more than that, the *A*-line mouse, when it grows up, will accept any later graft from a *CBA* donor as if it were its own. I shall begin by using the term immunological tolerance in the rather restricted sense that is illustrated by this experiment, and shall discuss its more general usage later on.

The experiment I have just described[1] can be thought of as an artificial reproduction of an astonishing natural curiosity, the phenomenon of red-cell chimerism in certain dizygotic twins. The blood systems of twin cattle before birth are not sharply distinct from each other, as they are in most other twins; instead, the blood systems make anastomoses with each other, with the effect that the twins can indulge in a prolonged exchange of blood before birth. In 1945, R. D. Owen[2] made the remarkable discovery that most twin cattle are born with, and may retain throughout life, a stable mixture—not necessarily a fifty-fifty mixture—of each other's red cells; it followed, then, that the twin cattle must have exchanged red-cell precursors and not merely red cells in their mutual transfusion before birth. This is the first example of the phenomenon we came to call immunological tolerance; the red cells could not have 'adapted' themselves to their strange environment, because they were in fact identified as native or foreign by those very antigenic properties which, had an adaptation occurred, must necessarily have been transformed. A few years later R. E. Billingham and I[3], with the help of three members of the scientific staff of the Agricultural Research Council, showed that most dizygotic cattle twins would accept skin grafts from each other, and that this mutual tolerance was specific, for skin transplanted from third parties was cast off in the expected fashion. We did not set out with the idea in mind of studying the immunological consequences of the phenomenon described by Owen; on the contrary, we had been goaded by Dr. H. P. Donald into trying to devise a foolproof method of distinguishing monozygotic from dizygotic twins, an enterprise that seemed reasonable enough against the rather thorough background of knowledge we already possessed about the behaviour of skin grafts in experimental animals[4]. It was F. M. Burnet and F. Fenner[5] who first read a general significance into Owen's discovery and who wove it into a general hypothesis of the immunological response which counted the phenomenon of tolerance among its theoretical consequences.

In the outcome, it proved impossible to distinguish between the two kinds of twins by skin grafting; but the causal connexion between Owen's phenomenon and our own was obvious, and we were now confident of our ability to make adult animals accept tissue homografts by reproducing in the laboratory the very state of affairs that had come about by natural accident in twin cattle. Billingham, L. Brent and I eventually succeeded in doing so, and our first report on the matter was published in 1953 [1]. In the meantime, M. Hašek[6], working against an entirely different conceptual background, had reproduced Owen's phenomenon in chickens by the ingenious method of making a deliberate synchorial parabiosis between chick embryos in the shell. At hatching the parabionts separated, and from now on they were incapable of making antibodies against each other's red cells, or, as later work showed[7], of rejecting grafts of each other's skin. It is now known that chimerism can occur naturally, though rarely, in twin sheep, and more rarely still in twin human beings[8]; in twin chickens it is probably the general rule. That chimerism should occur in man is clear proof that the principle of tolerance applies to human beings as well as to laboratory animals, and human chimeric twins can accept grafts of each other's skin[9].

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* Substance of Nobel Lecture delivered in Stockholm on December 12, 1960.

Some Properties of the Tolerant State

The main points that emerged from our analysis of the tolerant state were these. In the first place, tolerance must be due to an alteration of the host, not to an antigenic adaptation of the grafted cells, for grafts newly transplanted in adult life have no opportunity to adapt themselves, and the descendants of the cells injected into fœtal or new-born animals can be shown by N. A. Mitchison's methods to retain their antigenic power[10]. Once established, the state of tolerance is systemic; if one part of the body will tolerate a foreign graft, so will another; we found no evidence that a tolerated graft builds up a privileged position for itself within its own lymphatic territory. The stimulus that is responsible for instating tolerance is an antigenic stimulus—one which, had it been applied to older animals, would have caused them to become sensitive or immune. A plural stimulus can induce plural tolerance; the donor will usually contain several important antigens that are lacking in the recipient, and long-lasting tolerance must imply tolerance of them all. The state of tolerance is specific in the sense that it will discriminate between one individual and another, for an animal made tolerant of grafts from one individual will not accept grafts from a second individual unrelated to the first; but it will not discriminate between one tissue and another from the same donor. The injection of leucocytes or lymphoid cells can confer tolerance of skin grafts, for example; and later work has shown that the same is true of grafts of thyroid tissue, ovary, kidney and adrenal gland. These various tissues do indeed differ in their antigenic make-up, but not, apparently, in respect of antigens that play an important part in transplantation immunity. If leucocytes lacked some important antigen present in skin, it is difficult to see how they could confer tolerance of skin; and P. L. Krohn, using more direct and more critical methods, has found it impossible to distinguish between the antigenic make-up, so far as it governs their transplantability, of ovary and skin[11].

Tolerance can be brought promptly and permanently to an end by an experimental device which combines certain principles established by N. A. Mitchison and M. W. Chase. Consider the model experiment described at the beginning of this article, and imagine an *A*-line mouse which is tolerant of *CBA* tissue and which carries a *CBA* skin graft as outward evidence that it is so. The tolerated *CBA* graft can be destroyed within a week by injecting into its host lymphoid cells from *A*-line mice which have reacted on and rejected *CBA* tissues in the expected fashion. A less spectacular but in some ways more informative variant of this 'adoptive immunization' is to inject the tolerant mouse with lymphoid cells from *A*-line mice that have *not* been sensitized beforehand by *CBA* tissues. Here, too, though much more slowly, the tolerant state is brought to an end. The inference we drew from this experiment—and nothing has occurred since to make us question it—was that tolerance is due to a central failure of the mechanism of immunological response and not to some intercession at a peripheral level.

There is one important question about tolerance which the experimental system I have been describing is very ill-equipped to answer: Does the maintenance of the tolerant state depend on the continuing presence of the antigen that provoked it? When living cells are used to procure tolerance they survive into adult life and therefore maintain a chronic antigenic stimulus. But is it necessary that they should do so? So far as transplantation immunity is concerned, no completely confident answer can be given until the antigens that excite it can be extracted in a sufficiently potent form; but with 'tolerance' of foreign proteins and red cells, it does indeed seem that antigen must continue to be present, even though in quantities below the threshold of direct estimation, if a fully non-reactive state is to be maintained[12]. This is one of the most important single pieces of evidence that must be taken into consideration when devising hypotheses to account for tolerance, and it points to the clear distinction that must be drawn between the dosages of antigen necessary on one hand to instate tolerance, and on the other hand to maintain it. Much else is still uncertain—for example, the significance to be attached to the 'partially tolerant' state. Tolerance is not an all-or-nothing phenomenon: every degree of tolerance is to be found, from that which allows a graft to live just perceptibly longer than would be expected of it in a normal animal to that in which the graft is permanently accepted by and incorporated into its host. Is an animal partially tolerant because all its reactive cells are almost completely debilitated, or because, while most of them are completely out of action, a minority retain full possession of their powers?

Tolerance and Runt Disease

It has been the experience of all laboratories that tolerance is most easily procured by injecting lymphoid cells into immature animals. This is not because lymphoid cells are in themselves more strongly antigenic than others—there is evidence that they are not so—but probably because they are distributed throughout the body of the animal into which they are injected. This should make them adept at producing tolerance; an animal can be immune when only a minority of the cells that are competent to do so are reacting immunologically, but cannot be tolerant unless the very great majority of them are not. Unfortunately, the lymphoid cells used to induce tolerance are immunologically qualified to attack the tissues of their host, with consequences that were first revealed by Billingham and Brent[10] and, in a somewhat different form, by M. Simonsen[13]. The injection of foreign adult lymphoid cells into new-born mice of an unrelated strain gives rise to a fatal or chronic illness, 'runt disease', marked by retardation of growth and widespread damage to the host's lymphoid tissue. Its discoverers have proved beyond question that runt disease is immunological in origin. So far as it concerns mice, the clear recognition of runt disease had to await the development of a technique for injecting new-born mice intravenously[14]; until that had been done, there was every inducement to believe that death or stunting was due to accidental damage caused by injecting cells into mice *in utero*. Runt disease or splenomegaly (one of its earlier symptoms) provides a test of the immunological competence of cells, and lends itself to exact studies of their immunological capabilities; perhaps the most important discovery that can be credited to it is of the presence of an immunologically competent cell in peripheral blood. The aspect of runt disease which concerns us here is its relationship to tolerance. A state of tolerance must obviously abet the onset and probably the progress of runt disease, because if adult lymphoid cells are to attack the tissues of the animal into which they are injected they must live long enough to be able to do so. The frequency with which runt disease was associated with the induction of tolerance gave rise to the suspicion that tolerance might itself be a pathological condition. Might not the adult lymphoid cells used to procure it simply exterminate the developing lymphoid cells of the host and take their place? This cannot be so. Tolerance unaccompanied by any symptom of runt disease is produced by the injection of embryonic cells or by a natural or artificial parabiosis between embryos, and it leads here to a stable chimerism in which native and foreign cells seem to coexist without the one ousting the other. It can be produced, moreover, by adult lymphoid cells which, though antigenically foreign to their hosts, are for simple genetic reasons incapable of attacking them. The experiment described at the beginning is in fact best carried out by injecting, not adult *CBA* lymphoid cells, but adult lymphoid cells from a first generation hybrid between mice of strains *A* and *CBA*. By and large there seems no reason to believe that tolerance can be explained in terms of cellular competition and replacement.

Other Forms of Immunological Non-reactivity

The antigenic substances I have so far had in mind are 'foreign' only in the sense that they derive from other members of their recipients' species. Tolerance of more remotely foreign cellular antigens is more difficult but certainly not impossible to achieve. But even if we confine the concept to the reactions that take place within the compass of a species, it is clear that tolerance can be induced by antigens belonging to more than one chemical class and may extend to more than one modality of response. A tolerant animal fails not only to engage in the 'cellular' type of response we associate with the reaction against homografts of skin; it fails equally to make humoral antibodies—for example, *iso*hæmagglutinins; and the antibodies that fail to make an appearance include those of so-called 'natural' occurrence (for example, human anti-*A* or anti-*B*) as well as those that appear only in response to deliberate immunization. It is not surprising, then, that many authors[15] should have found that a state not yet formally distinguishable from tolerance should be brought about by the injection of purified protein antigens into young animals.

Even in this wider sense tolerance is by no means the only kind of specific immunological non-reactivity[16]. L. D. Felton described and named the phenomenon of 'immunological paralysis' in 1949. Felton showed that adult mice could be immunized and therefore protected against otherwise lethal doses of living pneumococci by the injection of 0·5 μgm. of a pneumococcal polysaccharide of the appropriate antigenic type. But, so far from conferring resistance, the injection of larger doses (500 μgm., for example) abolished it, for the mice became vulnerable to pneumococcal infection and could now no longer be protected against it by an injection of the smaller dose. An analogous phenomenon was discovered by F. J. Dixon and P. H. Maurer when they showed that large doses of soluble protein antigens failed to excite a response from adult rabbits. The foreign protein disappeared from the circulation at just the rate that would be expected if no antibodies against it had been formed. At one time most of us believed that immunological paralysis could not be classified as an essential non-reactivity. Antibodies were probably formed, we believed, but were promptly bound by antigen present in vast excess. But the most recent work, notably from A. H. Coons's laboratory, makes this interpretation rather doubtful, for refined methods have so far failed to reveal that any immunological reaction is afoot. These observations have given rise to the hope that paralysis and tolerance of soluble antigens may be indistinguishable phenomena. The problem now to be decided is whether or not the inception of tolerance in the narrower sense depends on some quantitatively distinctive property of immature lymphoid cells. Are embryonic cells specially easy to paralyse, for example, or, what comes to the same thing, is it specially difficult to make adult cells tolerant? I know that work bearing on this problem is in progress in Coons's and Mitchison's laboratories. For many reasons—one of them the need to distinguish between the doses of antigen needed for the inception and the maintenance of the unresponsive state—the problem is by no means so easy to tackle as might appear at first sight. It is a matter of some importance that a quantitative study of the same kind should be made of tolerance of foreign tissue grafts. If I may refer once again to the experiment described at the beginning, we can be sure that an adult *A*-line mouse will not be made tolerant of *CBA* tissues by the injection into it of a proportionate multiple of the number of *CBA* cells that would have sufficed if their recipient had been newly born; but we still do not know whether a disproportionately large number of *CBA* cells might not confer tolerance on a mouse, say, ten days old. However, the possibility that tolerance and paralysis are no more than quantitatively distinguishable, if even that, makes it more urgent than ever before to prepare the antigens that excite transplantation immunity in an adequately potent form.

Two other forms of non-reactivity should be mentioned. One is the specific tolerance of tissue grafts which may be conferred upon a totally irradiated mouse the hæmatopoietic tissues of which have been restored by an injection of foreign bone-marrow cells. J. F. Loutit has suggested that the phenomenon is akin to tolerance in the narrower sense defined by our own experiments, but here, too, the analogy with immunological paralysis is not far to seek. Curiously enough, the encouragingly long survivals of kidney homografts reported by our surgical colleagues in Boston and Paris[17] have been brought about by the use of rather lower doses of whole-body irradiation than experience with mice might have tempted one to regard as essential. Perhaps the behaviour of inbred and homozygous mice is not the best theoretical guide to what may be expected of animals so obstinately heterozygous as human beings. Is it possible that 'radiation induced tolerance' might be easier to secure when the mouse donors are hybrids of type X/Y and the recipients hybrids of type Y/Z? This model is also unrealistic, but more akin in principle to the genetic situation that human beings confront us with in real life.

Finally—I have put it last because it is the least easy to classify, though it might turn out to be the paradigm of all such phenomena—I must mention the inhibition of drug allergy described by M. W. Chase and M. Sulzberger[17]. Guinea pigs fed by mouth with, for example, picryl chloride, no longer develop a cutaneous hypersensitivity of the delayed type on the application of that substance to the skin. Analysis has shown that this, too, is an essential non-reactivity. Many of us nourish the hope that a similar principle might be turned to good account in transplantation immunity, if it should prove possible to prepare a 'haptenized' derivative of the antigens that cause it to come about.

Biological Significance

Tolerance is a natural phenomenon, though its natural occurrence among chimeric twins may be something of an oddity. Has it any wider zoological significance?

The immunological defences of the body are directed against foreign matter. It is not at all common for an animal to react on the native ingredients of its own body. Burnet was the first to realize that this is not a state of affairs to be taken for granted, but something that calls for a special explanation; and it was his attempt to explain it that led him to predict that antigens which impinged upon an animal sufficiently early in its life should come to be accepted as if they were its own. Now that tolerance is an established fact we may turn his argument the other way about. Tolerance is ideally well qualified to provide a built-in natural safeguard against the danger that an animal might be sensitized or immunized by the constituents of its own body, and this may turn out to be its biological *raison d'être*[18]. If this argument is true, then the only substances capable of exciting auto-immune reactions should be those which never normally have access to centres of immunological response, so that the body has no opportunity to learn to tolerate them. I do not know of any evidence that contradicts this inference.

Tolerance makes one think anew about the special relationship that holds between a mammalian mother and its unborn young. There are hints in the literature that a mammalian infant may be specially tolerant of antigens of maternal origin, and evidence of such a relationship should be found more readily among human beings than elsewhere; for unlike rats and mice we have a long gestation period, and unlike sheep and cattle our fœtal membranes are permeable by at least some molecules as large as proteins. Yet it seems most unlikely that maternally induced tolerance should be of regular or well-defined occurrence. The permeation of the fœtus by foreign antigens gaining access through the mother might weaken its resistance in later life to infectious disease (we may recall here the significance read by Burnet and Fenner into Traub's observations on lymphocytic choriomeningitis[5]). This is only one among half a dozen reasons why traffic between maternal and fœtal circulations should be under very close control. The occurrence of runt disease makes it, of course, unthinkable that the barrier between mother and fœtus should be easily penetrable by anything so large as maternal cells.

Theories of Tolerance

Burnet and I have agreed on a division of labour which absolves me from speculating on the causes of the various kinds of essential non-reactivity I have described. Tolerance, like the secondary response, and the nature of immunological 'memory', has become something of a testing ground for theories of the immune response. It must be said that the occurrence of tolerance does not yet follow from any hypothesis of the nature of the immune response that has been verified by *independent* means. Far too much is still uncertain. We do not yet know whether any one antibody-forming cell is potentially capable of making any antibody within the organism's immunological repertoire or whether the competence of any one such cell is restricted to a sub-class of the reactions that can be engaged in by the organism considered as a whole. We do not yet know whether the act of synthesis undertaken by an antibody-forming cell is strictly and specifically underwritten by the cell's genetic make-up or whether, in J. Lederberg's terminology, the instructions that govern that act of synthesis are imparted by the antigen itself. If it should be true that the antigen does no more than choose between one set of pre-existing instructions and another, we still do not know whether those instructions are already present in the zygote and therefore part of the legacy of its descendants, or whether they must be added to (by mutation, necessarily) during the course of growth. Finally, we do not even know whether the antitheses as I have put them are wisely so put or not. But it is the study of tolerance that has raised these questions in a specially urgent form[19], and which, in due course, will make an important contribution to their answers.

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NEWS and VIEWS

New Year Honours

THE following names of scientists and others associated with scientific and related work appear in the New Year Honours list :

Baron : Sir Alexander Fleck, chairman of the Advisory Council on Research and Development, Ministry of Power, and of the Nuclear Safety Advisory Committee, for public services.

C.H. : The Earl of Limerick, chairman of the Medical Research Council.

Knights : Dr. Christopher H. Andrewes, deputy director, National Institute for Medical Research ; Prof. Alfred C. B. Lovell, professor of radio astronomy, University of Manchester, and director of the Jodrell Bank Experimental Station ; Alexander Oppenheim, vice-chancellor of the University of Malaya ; John N. Ritchie, chief veterinary officer, Ministry of Agriculture, Fisheries and Food ; Dr. Alan H. Wilson, a deputy chairman and managing director of Courtaulds, Ltd. ; Prof. Brian W. Windeyer, professor of radiology, University of London, dean of Middlesex Hospital Medical School ; Dr. Alfred E. R. White, of Toorak, Victoria, Australia, for philanthropic services to the advancement of culture and the development of medical research.

C.B. : H. A. Sargeaunt, deputy chief scientist, War Office ; W. H. Stephens, director-general of ballistic missiles, Ministry of Aviation.

C.M.G. : W. F. Searle, chief statistician, Colonial Office ; Prof. S. Sunderland, professor of anatomy, University of Melbourne, chairman of the National Radiation Advisory Committee ; Prof. A. D. Trendall, master of University House and deputy vice-chancellor, Australian National University, Canberra

C.B.E. : J. B. Alexander, director of the Geological Survey, Federation of Malaya ; G. Burdon, engineer surveyor-in-chief, Marine Division, Ministry of Transport ; J. Cook, director of agriculture, Sarawak ; Dr. E. H. Derrick, deputy director, Institute of Medical Research, State of Queensland ; G. C. I. Gardiner, technical director, De Havilland Aircraft Co., Ltd. ; Prof. R. B. Green, emeritus professor of anatomy, and lately dean of medicine, King's College, University of Durham ; W. A. Grinsted, director of the West Indies Meteorological Service ; Prof. C. H. Stuart-Harris, professor of medicine, University of Sheffield ; G. K. B. Hay, formerly director of agriculture, State of Western Australia ; Dr. G. A. C. Herklots, principal of the Imperial College of Tropical Agriculture, West Indies ; F. T. Ingham, lately director of Geological Surveys, Cyprus ; Dr. D. Dilwyn John, director of the National Museum of Wales, Cardiff ; R. H. Jones, chief electrical engineer, Crown Agents for Oversea Governments and Administrations ; C. E. T. Mann, formerly director, Rubber Research Institute of Malaya ; W. J. M. Menzies, member of the Advisory Committee on Fishery Research ; Prof. J. C. Mitcheson, professor of mining, University of London (Imperial College, Royal School of Mines) ; Prof. J. A. Manché, vice-chancellor and rector magnificus of the Royal University of Malta ; E. F. Newley, deputy director, Atomic Weapons Research Establishment, Aldermaston ; A. A. Rubbra, technical director, Rolls-Royce, Ltd. ; G. C. Turner, a principal actuary, Government Actuary's Department ; E. J. Vaughan, a deputy chief scientific officer, Admiralty ; G. E. Watts, principal, Brighton Technical College ; Prof. A. W. Williams, professor of medicine, Makerere College, Uganda ; H. Woods, H.M. deputy chief inspector of factories, Ministry of Labour.

Biochemistry at Trinity College, Dublin : Prof. B. Spencer

DR. BRIAN SPENCER has been appointed to the chair of biochemistry at the University of Dublin, established in place of the chair held for his life-time by the late Dr. W. R. Fearon (obituary, see *Nature*, 185, 283 ; 1960). Dr. Spencer studied at the University of Liverpool and was awarded a B.Sc. with first-class honours in biochemistry in 1948, and a Ph.D in 1950 following postgraduate work with Prof. R. T. Williams at Liverpool and St. Mary's Hospital Medical School. Later, he joined the staff of the Department of Physiology, University College of South Wales and Monmouthshire, Cardiff, and in 1956 transferred to a newly created Department of Biochemistry under Prof. John Pryde. In 1956–57, Dr. Spencer spent a year's leave of absence working on biochemical cytology with Dr. E. V. Cowdry at Washington University Medical School, St. Louis. Dr. Spencer developed an interest in sulphate esters during his postgraduate work in the field of detoxication, and at Cardiff he became well known for his work on sulphatases and on sulphate ester synthesis. In recent years he has extended his interests to the biochemistry of epithelia and teratogenesis.

Bacteriology at the London School of Hygiene and Tropical Medicine : Prof. E. T. C. Spooner

PROF. E. T. C. SPOONER recently gave up the chair of bacteriology at the London School of Hygiene and Tropical Medicine to become full-time dean of the School. He was educated at Epsom, St. Bartholomew's Hospital and Clare College, Cambridge, where he gained a Foundation Scholarship, and was elected a Fellow in 1929. Apart from two years as a Commonwealth Fellow at Harvard (1929–31), he spent the best part of twenty years at Cambridge as tutor, and as demonstrator and lecturer under H. R. Dean in the Department of Pathology. Many probably expected him to live and die a Cambridge don. In 1947, however, he was called to the chair at the London School of Hygiene and Tropical Medicine, which he has held until the present time. His contributions lie within the field of virus diseases, including dog distemper and mumps, and of tetanus and other anærobe infections and of hospital cross-infection. During the Second World War he held a temporary commission and visited the Middle East to advise about hepatitis and other problems. His importance to medicine has particularly been that of the wise counsellor. He was a member of the Medical Research Council from 1953 until 1957 and has been a much valued member of a number of its committees From 1949–55 he was editor of the *Journal of Hygiene*.

Prof. D. G. Evans, F.R.S.

DAVID GWYNNE EVANS has been appointed to succeed Prof. Spooner in the University chair. He is a science graduate of the University of Manchester. He began work on bacteriology in Manchester under Prof. H. B. Maitland in 1934, devoting particular attention to whooping cough. His best-known work is in the field of whooping cough—the antigens of *Haemophilus pertussis* and their relation to immunity. In 1940 he joined Percival Hartley in the Division of Biological Standards at the National Institute of Medical Research at Hampstead, where he worked on the standardization of toxins, especially those of gas gangrene. In 1947 he returned to Manchester to become a reader in Maitland's department. In 1955 he returned to the National Institute at Hampstead when it became urgently necessary to devise means for ensuring the safety and potency of poliomyelitis vaccines. This, coming at the time of the "Cutter incident", was a difficult task; we owe much to Evans and his colleagues for the fact that the British polio vaccines are second to none. In 1958 he succeeded Miles, taking charge of the Biological Standards Division. The value of his work was recognized a year ago when he was elected a Fellow of the Royal Society.

The New York Academy of Sciences : President

Dr. Frederick Y. Wiselogle, assistant director in charge of chemical research in the Squibb Institute for Medical Research, New Brunswick, has been elected president of the New York Academy of Sciences. During 1948 Dr. Wiselogle was awarded the Presidential Certificate of Merit for his work on anti-malarial drugs.

New Honorary Life-Members

The following were elected to honorary life-membership of the Academy: Sir Edward Dodds, Courtauld professor of biochemistry, University of London; Sir Alfred Lovell, professor of radio-astronomy, University of Manchester, and director of the Jodrell Bank Experimental Station; Dr. Giulo Natti, director of the Institute of Industrial Chemistry, Polytechnic Institute of Milan; Dr. Franz Buchner, director of the Pathological Institute, University of Freiburg; Dr. J. J. Trillat, director of research at the National Centre for Scientific Research, Paris. The total number of honorary life-members in the Academy is limited to 100. With the election of these five, the total number of honorary life-members of the New York Academy of Sciences is now 85.

Awards

Isidor B. Laskowitz, 284 Eastern Parkway, Brooklyn, has been awarded the D. B. Steinman Award of 500 dollars for his description of a new concept for a simple landing aircraft incorporating high speed, stability and vertical take-off without the use of rotors. Dr. Minoru Tsutsui, of the New York University College of Engineering Research Division, has been awarded the A. Cressy Morrison Award of 400 dollars for his description of the principles governing coupling of molecules through stoichiometric and catalytic activity.

European Co-operation in Space Research

In reply to a question in the House of Commons on December 12, the Parliamentary Secretary to the Ministry of Aviation, Mr. G. Rippon, said, on behalf of the Minister for Science, that recent negotiations in Geneva had led to the signature by ten countries, namely, Belgium, Denmark, France, Great Britain, Italy, the Netherlands, Norway, Sweden, Switzerland and Spain, of an agreement setting up a Preparatory Commission to study the possibility of establishing a European organization for co-operation in space research. The Geneva Conference expressed the hope that the Preparatory Commission would consider the closest possible co-operation between any organization which might later be formed for space research in Europe and any collaborative organization between European States for the development of a launcher. Mr. Rippon added that Britain would keep in close touch about the matter with the Commonwealth and particularly with Australia.

Student Grants in Great Britain

In a written answer in the House of Commons on December 19, the Secretary of State for Scotland, Mr. J. S. Maclay, said that the revised scale of parental contributions for university students announced by the Minister of Education on December 1 would come into force in Scotland in October 1961, and would apply to university students, students at colleges of education and students taking advanced courses in colleges of further education. It is the Government's intention to have the new rates of maintenance allowances in operation not later than October 1962, but an interim improvement of Scottish allowances will be made in October 1961. Mr. Maclay said he would consult the local authority associations about the complex problems involved in putting these arrangements into effect, and also about the small number of further education students taking shorter courses with lower entrance qualifications who would not be covered by these arrangements. The Government also proposes to accept the Anderson Committee's recommendations that no deductions should be made from grants from public funds in respect of an award holder's personal income, including income from scholarships and prizes, up to £100 a year, and that there should be no restriction on the student's freedom of choice of any university in Great Britain.

In replying for the Government in an adjournment debate on State scholarships in the House of Commons on December 21, the Parliamentary Secretary to the Ministry of Education, Mr. K. Thompson, pointed out that the acceptance of the Anderson Committee's recommendation, that a student who obtained a pass in a minimum of two subjects at Advanced Level in a General Certificate of Education examination and was accepted by a university for a place should qualify for an award, should make a separate system of awards to ameliorate financial hardship unnecessary. Under the new system, the financial basis of the State scholarship system is removed. Moreover, the State scholars are not by any means the only honours students in the universities, and he believes that the incentive to get a place in a university in an increasingly competitive field will itself provide a stimulus to students to give of their best in their sixth-form days. The Government is now ready for discussions with the responsible authorities of the universities and colleges to see how far the new arrangements could help to ameliorate the ill-effects of the pressures which such competition imposes on sixth-form curricula, and is seeking opinions on some proposals advanced by the Secondary Schools Examinations Council. Mr. Thompson said that under the new system, benefactors might be encouraged to offer prizes at more of the civic universities, and this might help to diminish the attraction of Oxford and Cambridge for the best students. Further, while it is proposed that the technical State scholarships should disappear, the Government proposes to retain the mature State scholarships, mainly in their present form. He agreed with Mr. Irvine White that there are real advantages in British students following courses at colleges in other parts of the Commonwealth, and said it is hoped before long to announce a successful outcome to discussions with local authorities on Commonwealth scholarships and the tenure of awards in Commonwealth universities.

Service to Teachers

FROM time to time there has appeared in the columns of *The Schoolmaster*, the journal of the National Union of Teachers, a feature article entitled "Treasure Chest for Teachers". The purpose of this feature has been to offer information about associations of an educational nature, various embassies, consulates, and industrial organizations which are willing to supply certain services and teaching material free or at reduced rates for the use of schools and training colleges. From the number of inquiries about this material received at the headquarters of the National Union of Teachers and the organizations concerned, it became apparent that teachers and school librarians, in primary and secondary schools as well as in training colleges, were finding the feature sufficiently useful for it to be re-issued in permanent form. The National Union of Teachers has now issued the material as a booklet to all engaged in education who may find it useful (Pp. 94. London: Schoolmasters Publishing Co. 1960. 3*s*.).

Endorsed Certificates in Biology

RESULTS of examinations for endorsed certificates in biology are reported in the *Institute of Biology Journal* (7, No. 4; October 1960). In 1960, 106 candidates sat for examinations for the endorsed certificates at Ordinary Level. The examinations were taken in eleven colleges and 63 people were successful. The higher endorsed certificate papers were taken in seven colleges by 25 candidates, of whom 23 were awarded certificates. The technical colleges participating were Acton, Belfast, Bristol, Brunel, Cambridge, Ewell, Guildford, Heriot-Watt (Edinburgh), High Wycombe, Nottingham and Paisley (West of Scotland). Courses are now established at Stockport and Norwich City College.

Teaching Mathematics in the Junior School

MANY primary school teachers and lecturers who teach mathematical methods in training colleges and university education departments will welcome a recent publication by the National Union of Teachers (Mathematics in the Junior School. By J. S. Flavell. Pp. 28. London: National Union of Teachers, 1960). With the primary aim of making work understandable and so enabling the child to think, the booklet examines in detail the following fundamentals of a course outlined by Flavell: understanding of basic mathematical ideas, particularly number, quantity, size, notation and positional value; understanding of the meaning and significance of the fundamental operations of mathematics—addition and subtraction, etc.; the significance of zero; translation: this includes the ability to record in suitable form numerical relationships, the ability to translate from figure to word and *vice versa*, simple mathematical facts, statements and complete sentences (equations); generalization in mathematics; background, cultural studies of the growth through history of number ideas and practices, and of evolution of units of all kinds.

Journal of the International Union of Pure and Applied Chemistry

FOR many years now, decisions and recommendations made by the International Union of Pure and Applied Chemistry, particularly those on nomenclature, have been disseminated in the journals of participant countries. While this procedure has been reasonably satisfactory, the need for a more suitable medium of publication of these and other less-widely appreciated commission reports has become increasingly felt. At Paris in 1957 a Publications Committee was appointed to investigate the situation, and in 1959, at Munich, the Council decided to bring all its publications together in its own *Journal* under the guidance of an editorial advisory board. Because of the inevitable heterogeneity of the subject-matter to appear between the covers of this one journal, an arrangement was made whereby separate papers could be made available in pamphlet or book form. This first issue is largely concerned with publication of the proceedings of the symposium on "Radioactivation Analysis" held in Vienna during 1959, but also contains notes on the organization and functions of the International Union of Pure and Applied Chemistry and several reports. These reports describe: recommended standards for the microdetermination of carbon and hydrogen; the calibration of wave-length and photometric scales for non-recording spectrophotometers; the standardization of pH and related terminology; terminology of scales of working in microanalysis; terminology for use in relation to precision balances; preliminary recommendations on nomenclature and presentation of data in gas chromatography. Further issues are to deal *inter alia* with: dissociation constants for organic acids in aqueous solution; proceedings of the symposium on "Thermodynamics" (Firtzens-Wattens, 1959); proceedings of the symposium on "Tolerated Limits of Toxic Substances in Industry" (Prague, 1959). While one cannot but deplore the ever-increasing spate of scientific journals, the decision of the International Union to produce its own official journal should receive wide approval. It should be of great value to all chemists, and this first issue reveals that the *Journal* will indeed attract serious attention. It is understood that the *Journal* will be published at irregular intervals but will comprise four issues per volume. The subscription-rate is £6 per volume While no decision is announced, it would appear that the usual trilingual presentation in English, French or German is to be observed.

Bernice P. Bishop Museum, Hawaii

THE annual reports of many museums have recently been entirely re-cast, and in many instances are highly attractive and form interesting reading. The report for 1959 of the Bernice P. Bishop Museum, Honolulu, Hawaii, is entitled "Prelude to a Plan" and is an excellent example of the 'new look' in such statements (Pp. 32. Honolulu, Hawaii: Bernice P. Bishop Museum, 1960). The year was a prelude to a five-year plan for the whole Museum and covered such diverse aspects as the construction of a planetarium observatory, the inauguration of the Pacific Science Information Centre, and a retirement and annuity plan for the staff. The report shows clearly how the combined efforts of trustees, staff, volunteers and friends at home and abroad have carried the Museum through its seventieth year of service. The report is well illustrated with appropriate photographs of activities in the Museum.

The Water Research Association

THE period (1959–60) covered by the fifth annual report of the Water Research Association forms a milestone in the development of the organization for two reasons—it is the first year during which a grant has been received from the Department of Scientific and Industrial Research, and it is the last full year which the Association will spend in temporary accommodation at Redhill before moving to new laboratories at Medmenham (Pp. 24+4 plates. Redhill: The Water Research Association, 1960). A large part of the progress report of the Research Division, included with the Association's report, is concerned with laboratory experiments on factors affecting coagulation of suspended matter in water. At Medmenham, where a supply of water drawn from the Thames is available, it will be possible to carry out field trials on improved coagulation processes resulting from the laboratory work.

Problems of Ageing

BECAUSE little is known about the biological phenomenon of ageing and because of the problems which result from the increasing number of older people in the population, the U.S. National Institutes of Health have set up special programmes to stimulate and support research in all health-related aspects of ageing. As part of the research carried on within its own organization, the National Institutes of Health are conducting investigations into ageing in the Gerontology Branch of the National Heart Institute, located at the Baltimore City Hospitals in Baltimore, Maryland, and in the Section of Ageing of the National Institute of Mental Health, located in the Clinical Centre at Bethesda, Maryland. Within the research and training grant activity of the National Institutes of Health, there are many projects bearing directly on problems of ageing, ranging from investigations at the molecular level to investigations at the social level. Work on chronic disease in relation to the ageing process falls between these two extremes.

The Centre for Ageing Research, a component of the Division of General Medical Sciences, fosters additional investigations of all kinds of problems in ageing which are related to health. In addition to the research projects, both intra-mural and extra-mural, which are directly concerned with problems of the ageing process or ageing people, much of the research carried on or supported by the National Institutes of Health has an indirect or secondary bearing on problems of ageing. A booklet has now been prepared giving details of all the projects supported by the National Institutes of Health which have direct or indirect bearing on ageing (U.S. Dept. of Health, Education and Welfare: Public Health Service. Publication No. 761: Activities of the National Institutes of Health in the Field of Gerontology—Research Grant and Training Projects Active on January 31, 1960, and Intramural Research Projects carried on during calendar year 1959 (Washington, D.C.: Government Printing Office. Pp. v+41. 30 cents).

Current Medical Research

A VALUABLE document by the Medical Research Council contains reprints of the articles submitted by the Medical Research Council to Parliament for the year 1958–59 ("Current Medical Research". Pp. v + 56. (London: H.M. Stationery Office, 1960.) 3*s.* 6*d.* net). The subjects considered include the following: occupation and coronary heart disease; studies on the chemotherapy of tuberculosis in East Africa and India; clean air in operating theatres; human population genetics; chromosome abnormalities and disease in man; new applications of electron microscopy; the structure of viruses; the structure of muscle in relation to the mechanism of contraction; interferon: the prospects for an anti-viral agent in man; the arthropod-borne viruses; the plasma kinins and some factors in the problem of inattention.

Zoology of Persia

IT is, perhaps, surprising that the zoology of Persia is very imperfectly known: the country appears to have been neglected by Western zoological explorers, who have passed it by in concentrating on more distant lands to the east. A recent paper by Dr. Xavier Misonne describes the topography of Iran and the distribution of its mammalian fauna (Institut Royal des Sciences Naturelles de Belgique. Mémoires. Deuxième Série, Fasc. 59: Analyse Zoogéographique des Mammifères de l'Iran. Pp. 157+3 planches. Bruxelles: Institut Royal des Sciences Naturelles Belgique, 1960). Dr. Misonne was fortunate in being able to participate in the field investigations on the problem of plague transmission sponsored by the World Health Organization. These were particularly concerned with the distribution and biology of the rodents of the genus *Meriones*, to which the author gives special attention. As a result of several visits to the country, Dr. Misonne is able to give a comprehensive account of the distribution and ecology of the mammalian fauna, but he specifically disclaims that his work attempts a systematic revision of the Iranian species. His paper is illustrated with numerous distribution maps and several well-produced plates.

Tunnelling between Semiconductors

THE laboratories of both A. D. Little (Cambridge, Mass.) and the General Electric Company (Schenectady) have extended (*Phys. Rev. Letters*, 5, 461, 464; 1960) the latter's recent work on tunnelling at low temperature through a very thin insulator between two metals (*Phys. Rev. Letters*, 5, 147; 1960) down to temperatures at which both metals are superconducting. They prepared metal–insulator–metal sandwiches by evaporating a thin film of aluminium (transition temperature (T_c) of 1·2° K.) on to glass, exposing it to air to produce an oxide film about 20 Å. in thickness, and superimposing an evaporated layer of a second superconductor such as lead (transition temperature of 7·2° K.) or indium (transition temperature of 3·4° K.). The relationship between current (I) passed and voltage (V) applied across the sandwich then assumes particular interest below 1·2° K. at voltages around 1 mV., having a region for which dI/dV is negative—reminiscent of an Esaki (tunnel) diode for a voltage $\sim 0{\cdot}2$ V. Analysis of the relationship has enabled estimates to be made of the distribution of states of electrons in the super-

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conductors, and of the small energy-gap which modern theory postulates as a characteristic of superconductors; values of the gap of 4·3 kT_c and 3·6 kT_c are quoted for lead and indium, respectively. The region of negative resistance disappears when either metal of the sandwich is taken above its transition temperature; an applied magnetic field, by its control of T_c, can therefore modify the I/V relationship, and at 1° K. the current passed by one sandwich for an applied voltage of 0·2 mV. could be changed by a factor of 10^5. These effects thus make possible active devices capable of amplifying, and of acting as bi-stable switches.

The Torald Sollmann Award in Pharmacology

WYETH Laboratories of Philadelphia have established the Torald Sollmann Award in Pharmacology to commemorate the pioneer work in the United States of Dr. Torald Sollmann in the fields of pharmacological investigation and education. The Torald Sollmann Award consists of 2,500 dollars and a medal, and will be awarded for significant contemporary contribution to the advancement and extension of knowledge in the field of pharmacology. The Award is open to all scientists working in the field of pharmacology including, but not limited to, individuals from academic institutions, foundations, government and industrial and research organizations, regardless of age, sex or nationality. It will be given for originality and uniqueness of approach as well as development of the new concepts, theories and techniques which constitute a definite, mature and significant contribution to the extension and advancement of contemporary pharmacological knowledge. Not more than one Award will be made annually. Nominations for the Award must be submitted to the secretary of the Society (who is at present Prof. R. M. Featherstone, Department of Pharmacology, University of California, San Francisco 22) to be forwarded to the Chairman of the Torald Sollmann Award Committee not later than January 15 of each year to be eligible for consideration for an Award during the ensuing year. The first Award will be made at the 1961 autumn meeting of the Society in Rochester.

University News: Birmingham

THE following appointments have recently been made: G. R. Izaak, to be research associate in nuclear physics in the Department of Physics; Dr. C. F. Wells, to be lecturer in the Department of Chemistry; Dr. F. Bakhtar, to be lecturer in the Department of Mechanical Engineering; R. H. Hollier, to be Tube Investments Senior Research Fellow in the Department of Engineering Production; Mr. J. W. Fortey, to be lecturer in the Department of Civil Engineering; Mr. D. E. N. Davies, to be Associated Television Fellow in the Department of Electrical Engineering, Mr. B. Henderson, to be William Gibbins Research Fellow in the Department of Physical Metallurgy; Dr A. J. Rees, to be Imperial Chemical Industries Fellow in Geophysics, in the Department of Geology; Dr. C. E. Rowe, to be Caroline Harrold Research Fellow in the Department of Physiology

Edinburgh

THE following have been appointed to lectureships: Dr. T. D. Patten (engineering) and Mr D. C Malcolm (forestry). Dr. J. L. Holmes and Dr. A. J. Charlson have been appointed Imperial Chemical Industries Research Fellows for three years.

London

TITLES of professor in the University of London have been conferred on Dr. N. A. Barnicot (physical anthropology), in respect of his post at University College; Dr. L. F. Lamerton (biophysics as applied to medicine), in respect of his post at the Institute of Cancer Research: Royal Cancer Hospital. Titles of reader in the University of London have been conferred on Dr. R. H. Garstang (astronomy), in respect of his post at University College; Dr. M. K. Wells (geology), in respect of his post at University College. Dr. M. D. Milne, lecturer at the Postgraduate Medical School of London, has been appointed to the University chair of medicine tenable at Westminster Medical School.

The following appointments to readerships have been announced: Dr. F. A. Goldsworthy (applied mathematics), tenable at Queen Mary College as from January 1, 1961; Mr. E. J. Ambrose (physical chemistry), tenable at the Institute of Cancer Research: Royal Cancer Hospital; Dr. R. Creese (physiology), tenable at St. Mary's Hospital Medical School; Dr. L Miles Jackman (organic chemistry), tenable at the Imperial College of Science and Technology; Mr. M. J. Way (applied entomology), tenable at the Imperial College of Science and Technology; Dr. G. M. Timmis (chemistry), in respect of the post held by him at the Institute of Cancer Research: Royal Cancer Hospital; Dr. C. R. Webb (mechanical engineering), in respect of the post held by him at Queen Mary College: Dr. H. I. Winner (bacteriology), in respect of the post held by him at Charing Cross Hospital Medical School.

Announcements

SIR CYRIL HINSHELWOOD, Dr. Lee's professor of chemistry in the University of Oxford and past president of the Royal Society, has been awarded the Medal for 1961 of the Society of Chemical Industry. The Medal, a senior award of the Society, is presented every two years for conspicuous services to applied chemistry by research, discovery, invention or improvements.

DR. WILLIAM O. BAKER, vice-president of research at the Bell Telephone Laboratories, has been elected to the Board of Trustees of the Rockefeller Institute. Dr. Baker is also chairman of the National Science Information Council.

THE five hundred and seventy-seventh meeting of the American Mathematical Society will be held at Hunter College, New York, N.Y., on February 25 Further information can be obtained from Everett Pitcher, American Mathematical Society, 190 Hope Street, Providence 6, Rhode Island.

A SYMPOSIUM on "Temperature—its Measurement and Control in Science and Industry" is being jointly sponsored by the American Institute of Physics, the Instrument Society of America and the National Bureau of Standards. The symposium will be held at Columbus, Ohio, during March 27–31. Further information can be obtained from V. W. Sikora, Instrument Society of America, 313 Sixth Avenue, Pittsburgh 22, Pennsylvania.

THE FORM OF NEW UNIVERSITIES IN BRITAIN

THE second session of the Conference of Universities of the United Kingdom, held in London on December 10, discussed the problem of "New Universities".

In the first half of the present century the growth of the number of university students has been comparatively slow in Britain, and the creation of new university institutions has depended largely on local initiative and finance. The university colleges have had to depend mainly on students from their immediate region, and their academic staff have had to work to the curricula and examinations of their parent, the University of London.

In the past few years the situation has been radically transformed; the establishment of the University College of North Staffordshire, with frankly experimental ideas, set the tone, and considerations of national prestige, the popular demand arising from new prosperity, and the demands of industry and defence have all contributed to a change whereby new institutions can be established with almost 100 per cent State aid for buildings, equipment and salaries, and institutions which will be free to plan and control their own courses and which will be free to experiment as boldly as they wish. These institutions are receiving abounding goodwill and assistance, from the University Grants Committee, local authorities, industry, other cultural institutions, the general public and the staffs of existing universities through the Association of University Teachers.

The two main addresses of the conference had a contrasting form, that of Mr. J. S. Fulton, principal of the University College of Sussex, dealing with the solutions of the problems of siting, staffing, curricula and policy as they were being worked out in practice and planning, and that of Prof. L. C. Sykes, of the University of Leicester, who spoke of the underlying principles which should guide a new university.

Mr. Fulton presented three main topics for discussion, namely, siting, size and function. The first topic appeared to offer two solutions, apart from considerations of population distribution: either the new university should be placed in some town like York, where there are already many other cultural institutions, or in some area with few in the hope that it would become the focus of cultural growth. Size would depend on two considerations: a minimum number of teachers to cover essential subjects would be 300, with a staff–student ratio of 1 to 10; this would give a minimum student body of 3,000. The maximum number would depend on the extent to which teaching methods and residential provision would offset the social disadvantages of larger numbers. Mr. Fulton thought that new institutions should grow rapidly, and he hoped that Sussex would reach 3,000 in ten years.

Mr. Fulton said that the growth of the student population and the new careers open to graduates require a new approach to teaching; he asked if courses devoted primarily to the development of scholars are necessarily appropriate to the 'new student'. At Sussex they are planning courses on lines which owe something to the experience of North Staffordshire. The courses in science have yet to be planned in detail, but in arts they are proposing a combination of an introductory first-year course with an intensive study of a subject in, not a department, but a 'school'. At first there are to be schools of European studies, English studies and social studies, to be followed with a school of international studies. In science, they are considering following the Cambridge pattern, with two main subjects examined at the end of three years, with subjects like the history or philosophy of science, with a further year in one subject for specialists. He hoped to see the development of courses that would link the faculties, biology with psychology or sociology with applied science.

Finally, Mr. Fulton said that he considered teaching should be based on some kind of tutorial system, which creates a close personal relationship between the teacher and the student.

There was much agreement in principle in the second address, by Prof. Sykes; but he also raised matters which provided much of the framework for the discussion which followed.

Education as a preparation for life rather than primarily as a professional training should be the aim of a new university, and to achieve this the university should devote energy to preparing the student's mind to use his course before he left school This should be done through parents and teachers, as well as by means of mass media.

The problems of teaching for the citizens of the 'Affluent Society', who need more to control technology than to develop it, would depend on the attitude of mind of teachers. Teaching must no longer take second place in the interest of the academic staff, and research must no longer be the main criterion of academic worth. Successful teaching would depend in part on the creation of a community of learning in which domestic, architectural and landscape planning would be important, and in part on the development of a close personal relationship between the student and the teacher through forms of tutorial teaching. In the discussion that followed, these points were taken up by the Archbishop of York, Dr. Ramsay, who saw the aim of new universities as being the promotion of new knowledge as the prerequisite of the training of the imagination and the creation of a wholeness of understanding.

The question of the priority of teaching over research was a matter about which the conference disagreed, and both Prof. W. F. K. Wynne-Jones, of Durham, and Prof. P. J. Hilton, of Birmingham, argued that successful teaching depends on research, and that the two are not necessarily competing but complementary.

Another topic raised by Prof. Sykes which was widely discussed was that of the full participation of all the academic staff in the governing of the institution. He stated that if policy affects all, all should participate in its creation and that full information is the basis of loyalty.

This point was taken up in discussion by Prof. J. M. Cameron, of Leeds, who spoke of the importance of popular sovereignty and the dangers of oligarchy. Mr. T. G. Miller, of North Staffordshire, and Mr. H. Maddick, of the Association of University Teachers, supported these arguments, and the former urged the ending of the distinction between 'professorial' and 'non-professorial' teachers.

The problem of the independence of the new institution was raised by Prof. Sykes, and he emphasized the need for local financial and other support. In this he was supported with evidence by Prof. A. K. Stout, of Sydney, and Prof. J. E. Corry, the vice-principal of Queen's University, Ontario, both of whom dealt with some of the problems of political control. A reassurance was offered on this matter by Viscount Mackintosh of Halifax, who described the wide range of support given to Norwich by the University Grants Committee, by the general public, and by industry, commerce and the arts in the counties of Norfolk and Suffolk.

In contrast to the ideas of Mr. Fulton and in support of the thesis of Prof. Sykes, Prof. W. A. C. Stewart, of the University College of North Staffordshire, suggested that slow growth might in fact be the prerequisite of the development of successful inter-disciplinary courses, and he pointed out that his institution now has 800 students after ten years growth.

The sense of the conference was—it had no formal resolutions or conclusions—that education in the 'new universities' should be liberal rather than technical in the narrow sense, and should educate the whole man, or woman, for life rather than for a career.

IMPACT OF MAN ON THE VEGETATION OF THE HUMID TROPICS

An enterprising and successful symposium, convened by the Unesco South East Asia Science Co-operation Office, was held at Goroka, New Guinea, during September. It was brought to fruition through the generous hospitality of the Administration of Papua and the Territory of New Guinea and the unremitting efforts of J. S. Womersley, chief of the Division of Botany, Department of Forests, Lae. Thirty delegates attended from Australia, India, Indonesia, Malaya, Philippines, Sarawak, Singapore, Thailand, Great Britain, the United States, the Territory of Papua and New Guinea, and the South Pacific Commission. There were also twelve observers from the Territory, among whom Mr. H. P. Seale, district commissioner of the Eastern Highlands, was elected president of the symposium. Delegates arrived at Port Moresby, and on September 14 they were taken by charter-plane across the high mountains to Goroka at an altitude of 5,000 ft. Any doubts about the suitability of the site were soon dispelled by the comfort of the Goroka Hotel and the warm welcome from the district commissioner. In these inspiring surroundings, old in their display of the effects of early man in the valleys and on the mountain-sides, befitting the topic of the symposium, and new in the post-war growth of the administrative centre, a vigorous speech from the assistant administrator, Dr. J. T. Gunther, on behalf of the administrator, Brigadier D. M. Cleland, inaugurated the symposium. It was time, he said, to end not only the wars of man on man, but of man on beasts, and of man on vegetation. He struck a theme which forced itself into the chief recommendation of the meeting. The grandeur of Nature, the poverty of man, and the ideal of human rights surged into the panorama, for the object of the symposium was to bring together agriculturists, botanists, foresters, administrators, anthropologists and geographers in South East Asia and tropical Oceania.

Thirty-six papers had been presented and duplicated for previous study, and an efficient secretariat, brought from Port Moresby, reported the discussions in full. First, the physical and human backgrounds of the humid tropics were considered. Illness prevented Prof. W. A. R. Wikramateleke (Singapore) from giving the opening discourse, and in his place the aspects of climatology were presented by Prof. J. M. Blaut (Yale University), of soils and substrata by Dr. F. R. Fosberg (National Research Council, Washington), and of vegetation by myself. Prof. W. R. Geddes (University of Sydney) followed with a sympathetic approach to the human background, based particularly on his researches in Indo-China and Sarawak; he emphasized the 'c' in Unesco as the all-important socio-cultural element of human community and, indeed, the mark of man. The second topic, on the selection and cultivation of foods by early man, was introduced by Dr. J. Barrau (South Pacific Commission). He explained how early man was a practical geneticist selecting by taboo which prevented misappropriation and loss of valuable plants, thereby preserving the better yielders in the multitude of wild variety; the procedure was in striking contrast with the tendency to exterminate the valuable beasts. He was directing his studies to the problem of the bread-fruit tree, so widely cultivated in great variety, yet so obviously wild in New Guinea and the Melanesian islands. This important plant, too massive for interpretation in the herbarium, had to be studied in the arboretum, on which task he was bent. The problem of the sweet potato, chased by the peoples of New Guinea from valley to valley in almost maniacal destruction of the forest, raised considerable doubt on its supposed American origin. Fosberg presented his ideas on the origin of the coco-nut as a cultigen, the ancestral form of which botanists had not yet adequately contemplated. Mr. G. P. Keleny (Port Moresby) gave a detailed account of the basic food-crops of New Guinea. Dr. M. J. Meggitt (University of Sydney) explained his work on the native agricultural methods of the Western Highlands, showing the readiness with which new food-crops may be taken up. Mr. W. L. Conroy (Department of Agriculture, New Guinea), in comparing the effects of indigenous agriculture on soil-erosion and soil-deterioration with those of land-slides in forested country, considered that the damage supposed to be caused by deforestation might well be exaggerated. He instanced the value of *Casuarina* in re-afforestation and soil-renewal in the Highlands. Dr. E. Quisumbing (Manila) related how the War had caused many new food-plants to be taken up in the Philippines.

Thirdly came the prolonged discussion on the effects of fire. To most delegates the Highlands presented endless deforestation followed by grassland and incessant burning. They were grateful to Mr. C. F. W. Julius (Port Moresby) for his careful analysis of native land-rights and usage in explanation of this scenery. Achieved in the first place by finger-nails, sticks and stones, and now accelerated by trenching tools, knives and matchets, with a wanton love of burning for fun, yet there was little evidence in New Guinea of deterioration into desert conditions. Nevertheless, the question was raised whether the replacement of forest by grassland caused a lower rainfall, such as certainly followed the wake of the gold-dredging in the Bulolo district. The discussion revealed the abundance of observation, the thicket of conjecture, and the lack of scientific inquiry.

Fourthly, in an able discourse on grasslands, Dr. W. Bryan (Brisbane) opened the session on the effects of grazing by the domestic animals of early man. New Guinea was a great contrast to Africa. Deforestation led to grasslands, but the absence of any indigenous grazing animals was reflected even in the reluctance among natives to adopt cattle-farming. Though all became grass under native agriculture, there was so little flesh. Anthropologists joined with agriculturists in presenting this problem of induced pastoralism, extending into tropical Oceania, as a concern for Unesco. A remarkable paper on the status of the pig in New Guinea was read by Julius on behalf of Mr. T. Aitcheson (Port Moresby).

Fifthly, the effects of shifting cultivation on the vegetation of the humid tropics were considered. Mr. J. A. R. Anderson (Sarawak) described the carefully planned experiment on regeneration of cleared areas which was in progress in Sarawak. Contributions were made by Dr. I. Y. Carey (Malaya) on the methods of the Temiar people, by T. Smitinand (Thailand), E. Quisumbing (Philippines), Y. Satyanaryan (India) and D. J. Kingston (Port Moresby). What had been an economical cycle for soil-regeneration seemed now in all countries to be becoming uneconomical as increasing population shortened the fallow-period through the demand for more land in use. It was much regretted that no observer from the Food and Agriculture Organization had been able to attend this meeting.

Sixthly, there was a discussion on the nature of secondary plant-communities arising from the activity of early man. Fosberg introduced the subject with a general review of such communities. Dr. R. G. Robbins (Canberra) outlined the results of his researches on the New Guinea grasslands; the full publication of this work will be most instructive. Mr. J. J. Havel (Rabaul) gave two valuable papers on his experimental approach to forest regeneration in eastern New Guinea. Mr. E. C. G. Gray described the remarkably efficient sylviculture of mangrove-forest developed on their own by the natives in the Gulf of Papua.

Lastly, the social and economic aspects of early man's action on the forests were considered. Mrs. K. V. Bailey described the dire effects of over-population in the denuded limestone country of Java. Keleny described the land-use patterns in Java. Dr. I. Polunin (Singapore) discussed the aspects of human health and disease under conditions of shifting cultivation which tend to prevent intensification of parasitic attack.

This symposium touched on many vast problems, requiring the attention of both pure and applied science. The delegates were, indeed, grateful to Mr. L. Mattsson, director of the Unesco South East Asia Science Co-operation Office, for his help in enabling them to escape from their isolation in this huge region, to join in such fertile discussion, and to formulate in a set of resolutions to Unesco what they considered to be the most urgent matters for investigation. Herein they were assisted in no small measure by the hospitality of the Administration which had arranged two magnificent excursions by air and land-rover to enable them to see the natural vegetation, the grand mountain scenery, the happy native peoples and, indeed, the fine work of the Administration itself in the Highlands of the Territory. We flew in the early morning around Mt. Wilhelm (15,400 ft.) and its alpine slopes, glacial valleys, and many peaks in a cloudless sky. We picnicked in *Nothofagus* forests. We were the guests of the beautiful township of Kundiava in the Upper Chimbu. We visited the property of the Halstrom Fauna Trust at Nondugl, where birds-of-paradise are bred—and what an ornithological field-station it would make. Mr. Womersley took the botanists to Kainantu, whence they visited the agricultural station at Aiyura on the way to the scenic junction of the Ramu and Markham valleys, and they were taken along the great Markham valley in a spectacular flight to their destination at Lae. Dr. Meggitt took the anthropologists and geographers to the northern district of Madang. Some of us stayed on in the busy verdant town of Lae on the shore of the Huon Gulf and enjoyed the beautiful Botanic Garden, which is under the direction of Mr. Womersley. It was a delight to find this seat of learning. Started on August 1, 1949, from a waste of wild sugar-cane, it now covers some 120 acres of lawns, flower-beds, plant-houses, woodland, and wooded streams. The new office and herbarium, which are planned, will be the Mecca of Melanesian botany, and the next ten years should see the perfection of the first. Here, several of us, from different lands, walked and talked, consolidating our views on the symposium; as Fosberg sagely remarked, a requisite of a delegate to such a pioneering conference should be his ability to stay a while and contribute to the knowledge of the country.

The eight resolutions concerning South East Asia and Tropical Oceania directed to Unesco dealt with the needs for: (1) the study of secondary plant-communities produced by human disturbance of the tropical forest; (2) the promotion of ethnobotany; (3) the effects of alien civilization on primitive peoples; (4) the study of the vegetation of limestone formations, so susceptible to destruction by fire and other causes; (5) the urgent need for programmes of consideration of natural resources; (6) the study of root physiology in the furtherance of soil science; (7) research into the socio-economic adaptation of recently contacted native people; (8) the convening of another symposium within the region of the South East Asia Science Co-operation Office in view of the stimulus given already to scientific and cultural understanding by the previous ones.

E. J. H. CORNER

GAS CHROMATOGRAPHY

THE third international symposium on "Gas Chromatography" to be organized by the Gas Chromatography Discussion Group, this time in conjunction with the Society for Analytical Chemistry, was held at Edinburgh during June 8–10. More than 560 delegates attended the meeting, of whom more than 160 were from overseas. Three excellent receptions provided by the Organizing Committee, the Lord Provost of Edinburgh and the British Petroleum Co. were held in the evenings. Other items that added interest to the meeting were an instrument exhibition showing recent developments in commercial gas-chromatographic equipment, and visits to local industries arranged for the Saturday following the symposium.

The symposium was divided into three sections, Apparatus and Technique, Theory and Application, and General Applications, each being introduced by a well-known authority in the field. Preprints of the twenty-nine papers read were issued to delegates before the meeting, so authors had to point out only the highlights in their papers, thus giving more time for discussion. The full proceedings, edited by R. P. W. Scott, will be published by Butterworths Scientific Publications later.

The symposium began with a welcoming address by Prof. E. L. Hirst, of the University of Edinburgh, who described the impact chromatography had made on research in recent years. Dr. A. T. James, chairman of the Gas Chromatography Discussion Group, gave the opening address, outlining some of the more significant lines of research being currently followed. A report on the recent activities of the East German Working Group was read by Mr. H. P. Angele.

In the introductory lecture to the first session, "Applications and Technique", R. P. W. Scott pointed out areas where there was still much controversy among gas chromatographers. He emphasized the need for further work on column design, detectors and recording methods to improve both the speed and accuracy of quantitative analysis.

The recent very sensitive hydrogen flame and argon ionization detectors were the subject of the first session. L. Onkiehong and D. H. Desty reported investigations carried out on the flame ionization detector to determine the optimum design and operating conditions. Both concluded that the construction of the detector was not too critical except where the highest sensitivity is required. Similar results were found by R. C. Condon, who in addition compared the two ionization detectors. J. E. Lovelock investigated the design and operating parameters of the family of argon detectors. The triode detector, the most recently introduced, has a sensitivity of 10^{-14} gm./sec., about a hundred times better than that reported for the flame ionization detector. The sensitivity of all the argon detectors was found to be considerably reduced by the presence of trace amounts of hydrogen, nitrogen, oxygen, carbon dioxide and water in the argon. A comparison of integral and differential ionization detectors was made by S. Matousek.

Most of the discussion following the papers centred around the flame ionization detector. Many workers gave details of their own particular design, and it appeared that thick-walled jets which remained cool were preferable to those that glowed at red heat. F. van der Craats described how the linearity of the detector could be improved by using a cage rather than a disk as the upper electrode, and D. H. Desty stated that a small increase in sensitivity could be obtained by the addition of argon, nitrogen or oxygen to the hydrogen supply.

A disadvantage of the hydrogen flame-ionization over the argon detector is that an expensive impedance converter must be used to measure the low ionization currents produced. L. Onkiehong suggested that it may be possible to overcome this by the use of an a.c. polarizing potential (~ 100 c.p.s.) applied between the jet and a third electrode situated between the jet and collector. By this means a pulsed d.c. signal would be obtained at the collector which could be amplified by conventional methods. J. F. Smith gave details of an inexpensive amplifier using a cathode follower that was adequate where the highest sensitivity was not required.

An important feature not given sufficient attention by the majority of workers investigating detector performance was the determination of response factors for compounds of different type, to see whether any correlation exists between response and molecular structure. Difficulties in obtaining low known concentrations of vapours may be partly responsible for this, and the method for obtaining accurate concentrations down to 10^{-8} molar described by D. H. Desty should enable fuller work to be carried out in the future. Although no simple correlations were found in the papers read, in the discussion R. C. Condon pointed out that for the series of halogen compounds mono-, di- and tri-chloromethane the response of the flame ionization detector was proportional to the amount of hydrogen in the molecule, and F. Schild had found that the weight response for a number of oxygenated compounds was proportional to the product of the peak area and molecular weight. Both H. M. McNair and F. van der Craats compared the response factors that they had determined for hydrocarbons with those obtained by D. H. Desty and L. Onkiehong. A wide variation in relative response factors was apparent, and it may well be that these factors depend very much on the design and conditions under which the detector is operated.

The question of detectors of low concentrations of permanent gases arose, and T. B. Kent described how by using argon containing a few p.p.m. of acetylene as carrier gas concentrations of permanent gases in the p.p.m. region could be detected by the decrease in background signal produced. The use of the gas-discharge tube for the determination of small concentrations of impurity gases in argon was the subject of a paper read by B. Riley, and several industrial applications for an instrument of this type were outlined.

Following the discussion on detectors, attention turned to other developments in apparatus and technique. J. Boeke described the use of a modified form of frontal analysis in which the sample is continuously introduced into the column considerably diluted in an inert carrier gas, and gave details of a detector arrangement that enabled curves approaching differential rather than the usual integral records to be obtained. I. Halasz described an integrator based on a new principle having a count-rate of 1·8 million pulses per minute for a full-scale recorder deflexion and which may be adjusted to correct automatically for the calibration factor of the substance detected. A comparatively inexpensive high-speed recording system, using a long-persistence cathode-ray oscilloscope, was described by R. P. W. Scott, and its value for recording fast separations illustrated by several traces showing separations carried out in less than 1 min.

The next section, "Theory and Application", was introduced by M. J. E. Golay, who discussed the relative importance of resistance to mass transfer in the liquid and gas phases with packed and capillary columns. He described how it should be possible to obtain lower height equivalent to a theoretical plate values by increasing the surface area of capillary columns without increasing the distance across which the solute molecules have to diffuse. This could be achieved by covering the inner wall of a capillary column with a finely divided clay before coating, and the improvement was demonstrated by showing a peak obtained for benzene before and after treating a column in this manner.

Capillary columns were the subject of the first two papers in this section, and investigations of the important operating and design features for achieving high efficiencies and high speed of analysis were reported by R. P. W. Scott and A. Goldup. Both discussed the effect of column diameter, film thickness and carrier gas on the height equivalent to a theoretical plate linear gas velocity curve and compared experimental results with those predicted by the Golay theory. R. P. W. Scott was able, by using carrier gases of different diffusivities, to show experimentally that, in accord with theory, the contribution from resistance to mass transfer in the gas phase to the total height equivalent to a theoretical plate was greater than that due to mass transfer in the liquid phase for solutes that are well retained. In general, a fair agreement between theory and experiment was found at gas velocities corresponding to the minimum height-equivalent to a theoretical plate, but at higher velocities the agreement was less satisfactory. M. A. Khan suggested that this may be due to the absence in the Golay equation of a term accounting for interfacial resistance between the gas and liquid phases, and reported that he had recently published a note[1] discussing this effect.

Examples of the efficiency and speeds of analysis achieved on capillary columns were reported by A. Goldup. Using a 600-ft. glass column, an efficiency of 300,000 plates had been obtained for a well-retained solute, and the separation of sixteen out of a blend of seventeen octane isomers was given as an illustration of the resolution attainable. The potentialities of capillary columns for high-speed analysis were demonstrated by chromatograms obtained using a recording system having a time constant of only a few milliseconds, which showed the separation of a blend of nine heptane isomers in 58 sec. and a simple four-component blend in 1 sec. C. P. Quinn presented equations derived theoretically defining the best conditions to achieve the minimum time of analysis and supported these with experimental data. The basic premise of using linear gas velocities higher than the optimum was criticized by J. H. Knox.

In the first of the two remaining papers of the morning session S. J. Hawkes discussed factors which limit the maximum operating temperature of stationary phases. Valuable data on several stationary phases were given, and in addition the use of substituted borazoles at high temperatures was suggested.

Structural analysis of hydrocarbons by capillary gas chromatography in connexion with the methylene insertion reaction was the subject of a paper presented by M. C. Simmons. The author described how by simply treating paraffin hydrocarbons with diazomethane under ultra-violet irradiation a mixture of homologues of one higher carbon number could be obtained, and illustrated the value of this for obtaining retention volume data on inaccessible hydrocarbons. For example, retention volume results had been obtained for nineteen branched nonanes from six readily available octanes. One particularly interesting separation reported by the author was that of the diasterioisomers of 2,3,4-trimethylhexane. In the discussion that followed, J. F. Smith described the separation of an alcohol and a sulphide into *meso-* and *dl*-forms by gas chromatography.

Progress on large-scale preparative columns was also reported at the symposium. F. H. Huytens described how the loss in efficiency observed with preparative columns of large diameter could be attributed mainly to column packing. By carefully filling columns so that radial diffusion is large and axial diffusion small, height equivalent to a theoretical plate value of 2 mm. could be obtained with 3-in. diameter columns capable of handling samples up to 1 gm. The use of preparative scale chromatography for examining complex organic materials was reported by T. Johns.

Details of an apparatus for obtaining accurate retention volume data were given in a paper presented by E. R. Adlard. Specific retention volumes for *cyclo*hexane and benzene on dinonylphthalate at temperatures from 48° to 110° C. were used to determine excess partial molar thermodynamic functions. For both systems investigated the excess partial molar entropies were found to be the predominant cause of the departure from ideality. In an interesting contribution to the discussion, J. C. Winters described some results obtained by R. L. Martin in which marked changes in the specific retention volume for hydrocarbons on polar stationary phases had been observed. To account for this, R. L. Martin had suggested that adsorption of the solute on the surface of the stationary phase contributes to the retention of the solute as well as solution in the stationary phase, and had shown that the former becomes of increasing importance as the percentage of stationary phase decreases.

The final papers presented in the section "Theory and Application" were by C. G. S. Phillips and his co-workers. They described the use of transition metal complexes and saturated solutions containing free undissolved tri-*o*-thymotide as stationary phases. The latter compound is of particular interest since it forms inclusion compounds with molecules of restricted width. By comparing retention data obtained for tri-*o*-thymotide with that for di-*o*-thymotide which does not form such complexes it was shown that the former selectively retards straight-chain compounds. The application of complexes of this type to give a separation according to molecular shape appears to be a most promising extension of gas chromatography.

The final day, devoted to "General Applications" of gas chromatography, was introduced by A. I. M. Keulemans, who described some of the results, using chromothermography, obtained by Prof. A. A. Zhoukhovitsky in the U.S.S.R., By passing a ring furnace along the column during elution of the components it is possible to obtain symmetrical peaks using gas–solid chromatography at high sample concentrations. The technique enables samples of wide boiling-range to be examined, and has the added advantage that the temperature gradient and speed of the furnace offer additional parameters for controlling the separation.

The morning session was concerned mainly with the gas chromatography of inorganic compounds. Following a paper by G. Freiser describing the separation of the volatile chlorides of tin, titanium, niobium and tantalum at 200° C. on a 'Chromosorb' column coated with squalane, A. I. M. Keulemans mentioned that he had also separated volatile metal chlorides on an aluminium chloride-coated carbon column at temperatures up to 350° C. Both W. W. Brandt and J. Janak reported separating metals in the form of their acetylacetonates.

The chromatography of corrosive inorganic halogen compounds was the subject of two papers. T. R. Phillips employed a polytetrafluoroethylene capillary column coated with 'Kel–F' oils to separate mixtures of hydrogen fluoride, chlorine and chlorine trifluoride. A hydrogen-flame ionization detector, in which a platinum ring was maintained at red heat in the lower edge of the flame, was found suitable for detecting such inorganic compounds. Details of an apparatus incorporating a katherometer and automatic sampling valve, developed over a number of years for the analysis of plant streams containing highly reactive inorganic halide vapours, were given in a paper by G. Iveson.

Many other new and interesting applications of gas chromatography were described. J. Tranchant gave some results on the use of gas–solid chromatography for the qualitative and quantitative analysis of mixtures containing nitrogen oxides, and D. W. Hill described the application of gas chromatography to anæsthetic research. An illustration of how detailed information on gasolines may be obtained by a combination of liquid–solid and gas–liquid chromatography was illustrated by a number of chromatograms in a paper presented by S. T. Griffiths. C. G. Scott gave details of a method for investigating the stability of white oils. The oxidation products of low molecular weight are eluted with a stream of nitrogen, condensed in special traps and examined chromatographically.

In a discussion in which retention volume data were mentioned, J. F. Smith outlined a useful practical method for expressing all retention volume data relative to one standard 'theoretical' nonane.

In connexion with work on compounds of high molecular weight, J. Janak described how the composition of non-volatile materials could be investigated by the gas-chromatographic analysis of their pyrolitic products. The use of esters as high-temperature polar stationary phases for examining products encountered in polymer production was discussed by R. E. Roland-Jones, and two materials, polyethylene glycol adipate and ethylene glycol distearate, were found suitable for use at 250° C. C. G. Scott stated that he had been able to elute hydrocarbons containing up to fifty carbon atoms using alumina modified by treatment with alkali metal hydroxides as the column packing.

In the final paper of the meeting, G. R. Boreham described an apparatus suitable for fuel-gas analysis, which had as a special feature a multicell thermal conductivity cell that enables four columns to be operated in series with a detector at the outlet of each.

The meeting ended with an excellent summing up of the symposium by C. S. G. Phillips and a closing address by R. C. Chirnside, president of the Society for Analytical Chemistry.

A. GOLDUP

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[1] Khan, M. A., *Nature*, 186, 800 (1960).

ION EXCHANGE

ON December 2, the Permutit Co., Ltd., demonstrated a new film entitled "An Introduction to Ion Exchange". The programme was introduced by Mr. Walters, who gave a brief outline of the development and uses of ion exchange procedures, mentioning in particular the preparation of acid-resistant anion exchange resins. The film, which is in colour, commences by illustrating some of the uses of ion-exchange resins, including the production of potable water from sea water. On the molecular scale, the structure of polystyrene-based cation and anion exchangers is demonstrated using conventional atomic models, with the exchange process simply illustrated. On the macroscopic scale, the hydrophilic nature of dehydrated resin beads is shown by wetting them, followed by observation of the resultant swelling.

The three main operations of ion-exchange are then examined: (*a*) *Separation*. Here two coloured organic ions in solution are allowed to flow through a resin column. While the blue concentrates in a narrow band near the top of the column, the red is less strongly held, forming a wider band which could be eluted. (*b*) *Substitution*. The effect of passing a solution of potassium permanganate through a resin column in the chromate form is shown by a change in the colour of the solution from purple to yellow. (*c*) *Removal*. In this case a solution of a salt with two coloured ions, copper dichromate, is allowed to flow through a mixed-bed exchanger, resulting in the production of colourless 'de-ionized' water.

The exchange process is followed in more detail by a combination of demonstrations using copper dichromate and animated diagrams of sections through a resin bead in a flowing solution. A final survey of industrial aspects of water treatment completes the film.

Colour cinematography is an ideal medium for showing ion-exchange experiments, and in this sense the film is entirely successful. Nevertheless, although an attempt is made to discuss the mechanism of ion-exchange, a more critical and scientific approach would have been desirable. In particular, specific examples, in which the equilibria involved were considered in terms of quantitative data, would have been valuable. Perhaps the most elegant use of ion-exchange is in the separation of the lanthanide and actinide elements. This aspect is almost completely ignored. Again, although emphasis is laid on the low conductivity of 'de-ionized' water, particularly by comparison with distilled water, an indication that the conductivity of laboratory distilled water is due almost entirely to dissolved carbon dioxide and ammonia would not have been out of place. Further, the 'de-ionized' water contains significant quantities of organic impurities, some of them surface-active and not detected by conductivity measurements. The film will certainly appeal to many potential users of ion exchange; whether it will appeal to students and research workers remains to be seen. "An Introduction to Ion Exchange" is a 16-mm. film with an optical sound track, and runs for 27 min. It is available on free loan to learned societies, colleges and schools, industrial associations, etc.: applications should be sent to the Permutit Co., Ltd., Permutit House, Gunnersbury Avenue, London, W.4.

SERVICE WITH OVERSEAS GOVERNMENTS

NEW arrangements for service with overseas governments are detailed in a White Paper issued by the Secretary of State for the Colonies*. For reasons indicated in an appendix, the British Government has rejected as impracticable the idea of an Overseas Service based on the United Kingdom. Such a Service would not solve the problem of the future prospects of members of the Overseas Civil Service, but the Government is examining the feasibility of establishing a Commonwealth Advisory and Technical Service as suggested by the Select Committee on Estimates. Under the new arrangements the Government will assume full responsibility for the cost of the inducement element, which is a necessary part of the emoluments of most expatriate officers, and a fresh review of the emoluments of the officers concerned will be necessary in many territories. For purposes of United Kingdom taxation, the additional pension payable to an expatriate officer, which stems from the inducement allowance, will be treated in the same way as existing pension, and the cost of that part of the pension will be borne by the Government. The Government will pay education and children's allowances similar to those payable to members of the Home Civil Service posted overseas, and will also meet half the passage costs required to enable children being educated outside the territory in which an officer is serving to visit their parents, or to be visited by their mother, once a year. The Government has also contributed to some compensation schemes now in force for loss of career on premature retirement, or taken account of their cost in considering what further financial assistance should be given, and for future schemes will share the cost equally with overseas Governments.

The scheme will come into effect in most territories on April 1, 1961, and will cover up to 21,000 pensionable and contract officers overseas at a cost to the British Government of £12–£16 million a year. Besides all existing Colonies and dependencies, it will be offered to Nigeria, Sierra Leone and Singapore, and to Northern Rhodesia and Nyasaland, but not to the Bahamas, Bermuda, Brunei and Hong Kong, where, for several reasons, this form of help is not regarded as appropriate, at least for the time being. The arrangements have been developed after consultation with overseas governments and staff associations and they are complementary to plans being vigorously pursued for staffing the public services by local officers in the territories concerned. The White Paper also emphasizes the value of overseas officers in technical assistance schemes, and a high-level technical assistance working party has been set up under Foreign Office chairmanship as a means of exchanging information and experience between departments and organizations engaged in technical assistance work. A sub-committee of this body will co-ordinate the recruitment activities of the Government for technical assistance posts, and the fullest consideration will be given to retiring and retired members of the Overseas Civil Service for all suitable vacancies. It is also proposed to give greater publicity to the Overseas Services Resettlement Bureau, which has already placed more than 1,000 of the 1,710 officers registered with it from July 1957 to mid-October 1960; it is pointed out that the Bureau could prove a very valuable source of man-power to industry and commerce, particularly to export industries seeking men and women with long and detailed knowledge of overseas territories.

As regards the future, the proportion of candidates appointed on contract terms through the Colonial Office has increased markedly in recent years and such appointments now constitute the great majority. Wherever possible the British Government plans to obtain recruits on loan from government departments, public corporations and local authorities, so that individuals can be certain of resuming their previous employment on returning to the United Kingdom. It is also hoped that individual service overseas will be accepted as an asset both to the employer and employee, and that employers will co-operate in facilitating the release of those able and willing to serve overseas and in re-absorbing them on their return.

Further to the above, the Colonial Office has now issued the report of the Public Services Conference held in London during March 1–10, 1960, to exchange information on measures taken or projected to build up local civil services in the African territories, which was initially a confidential document for consideration by the governments represented at the conference*. All, however, have since agreed that it should be published, and it has now been issued by the Colonial Office.

The agenda covered the general pooling of experience and a comparison of the measures adopted; a review of the facilities for secondary and university education, including a comparison of scholarships schemes, training programmes; the exchange of information; the impact of localization on serving overseas officers; and the staffing problems which arise from the development of ministerial systems. The report makes no specific recommendations for governments to adopt, but indicates the important problems and the lines on which they might be tackled, leaving it for each government to reach its own decision after examining the report in the light of its own local conditions.

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* Colonial Office. Service with Overseas Governments. Pp. 22 (Cmnd. 1193.) (London: H.M. Stationery Office, 1960.) 1s. 3d. net.

* Colonial Office. Report of the Public Services Conference held in the Colonial Office, London, 1st to 10th March, 1960. Pp. iii+25. (Colonial No 347.) (London: H.M. Stationery Office, 1960.) 3s. 6d. net

The report suggests that each government should make a general review of the whole range of problems arising from the need to staff the local civil service increasingly with local people and that standing arrangements should be made to keep under consideration the policy as it develops. Discussing the provision of sufficient numbers of educated men and women, the need to provide secondary and higher education before satisfying all the needs of primary education was emphasized at the conference, and it was urged that the balance of secondary and higher educational systems should be planned on the basis of regular authoritative surveys of the estimated needs for educated man-power undertaken by really first-class experienced persons. As regards courses of training in the United Kingdom, which in 1959 provided for some 2,300 places, more than half of which were taken by local Civil Service officers, the Colonial Office stated that it was very ready to consider the provision of new courses or *ad hoc* training attachments to meet the requirements of overseas governments. In regard to the training of technicians and especially the training of supervisors in technical work, the conference agreed that no good answer had yet been found to the problem of training supervisors rapidly, and that there was no real substitute for experience on the job. The Colonial Office agreed to undertake the preparation of periodical reports directing attention to any new developments of interest or importance, including lists of subjects for which courses were run by overseas governments, the first such report to be prepared in about 18 months. In discussing the special staffing problems which arise from the development of the ministerial system and, in particular, its bearing on the tripartite relation between minister, permanent secretary and heads of departments, stress was laid on the importance of enabling professional officers to contribute fully in the formulation of policy and of respecting their professional integrity. It was also agreed that the professional branch of the Service should have the primary say in assessing the qualifications of the professional staff and their deployment. It also recognized that it was essential to build up proper administrative backing for the professional work, and in particular to ensure good staff relations with professional officers in the field when establishment work was undertaken by administrative and executive officers at headquarters.

FIELD-ALIGNED IONOSPHERIC IRREGULARITIES AND THE SCINTILLATION OF SATELLITE RADIO TRANSMISSIONS

By D. G. SINGLETON, G. J. E. LYNCH and Dr. J. A. THOMAS

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MANY investigations have been made of the nature of the signals received from artificial Earth satellites[1]. These have shown that the amplitude of the signals may vary as the result of one or more of the following four causes: (*a*) modulation of the satellite's transmitter; (*b*) rotation of the satellite antenna relative to the receiving antenna; (*c*) Faraday fading; (*a*) fading resulting from propagation through an irregular ionosphere. Suitable instrumentation can usually remove the results of cause (*a*), while causes (*b*) and (*c*) together give rise to a regular fading component with a period of several seconds[2] and can thus be recognized easily. The rapid and random fading is attributed to irregularities in the ionosphere, much the same mechanism being involved as is considered to be associated with the scintillation of radio stars.

Radio-star scintillations have been shown to be due to the passage of the radiation from the extra-terrestrial source through an ionosphere which has clouds of enhanced ionization embedded in it[3]. Further, these clouds have been shown to have dimensions of the order of a kilometre and to be elongated along the direction of the Earth's magnetic field[4]. There is considerable evidence, from ground-based radar investigations[5] of the ionosphere, which also points to the existence of such field-aligned irregularities. Recently, Rush and Colin[6] have considered the effect this anisotropy of the irregularities has on their ability to produce scintillations. They came to the conclusion that for propagation approximately along the field lines the scintillation activity normally associated with irregular diffraction by the irregular medium should be increased because of the possibility of radiation which traverses two completely different paths reaching the receiver. The first of these paths is the direct one; the second is via grazing reflexion from the field-aligned irregularities. The geometry which they considered applies to parallel radiation from an extra-terrestrial source, but a similar effect would be expected to occur when the source is rather closer to the Earth's surface.

Observations of the radio transmissions of satellite 1959 Iota-1 (*Explorer VII*) made at Brisbane (dip. 57°) during the months of July, August and September 1960, have been analysed in an attempt to establish the existence of this effect. The satellite takes approximately 8 min. to pass over the station and a scintillation index (fading amplitude/mean level) determined from the random fading component was allotted to each half minute of this period. From the known elements of the orbit it was possible to calculate, for each traverse, the time when the satellite passed through the observer's magnetic meridian plane. The scintillation index for the ½-min. containing this time was then normalized according to the

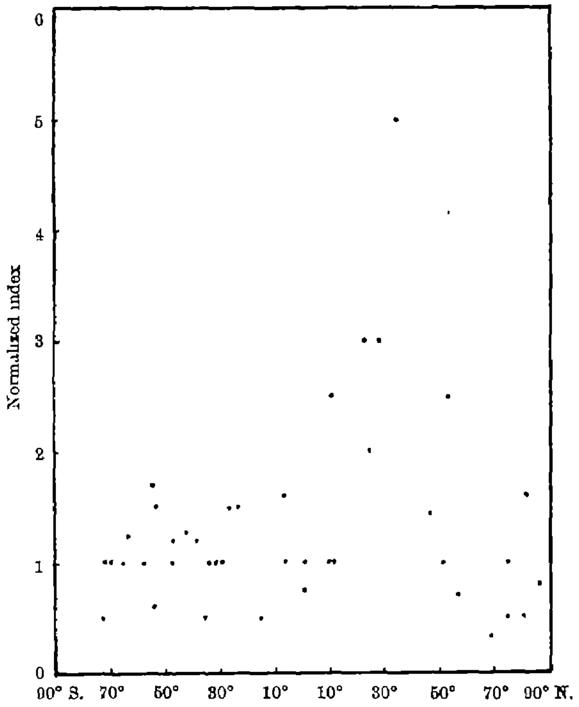

Fig. 1

general level of the scintillation index over the whole traverse. This was done to remove the diurnal and seasonal variations of scintillation activity.

A plot of the normalized scintillation index versus the zenith angle of the satellite as it passes through the magnetic meridian plane is shown in Fig. 1. If the scintillation activity is independent of the direction of propagation through the ionosphere, the points on this plot should be randomly distributed about a horizontal line, corresponding to a normalized index of one. While this is found to be generally true, the normalized index increases markedly over the range of zenith angles of 10°–55° to the north, being a maximum of 5 at 33°. This value of zenith angle in the magnetic-meridian plane corresponds to propagation along the field lines. The mean normalized index of the set of points in Fig. 1 is 1·33 with a standard deviation of 0·87. Consequently the observation at 33° is four standard deviations removed from the mean and thus constitutes an event, the probability of the occurrence of which by chance is 1 in 10^4. The two points corresponding to a normalized index of 3 are two standard deviations away from the mean, and the probability of their being chance events is 1 in 50.

Increases in scintillation index are occasionally observed when the satellite is at points outside the magnetic meridian plane. An analysis of all index increases above the general level has therefore been made to see if the increases associated with propagation approximately along the field lines are any different from the others. The number of increases for the various values of normalized index are listed in Table 1. Two of the three occasions when the normalized index is between 3 and 3·4 correspond to propagation approximately along the field lines, while the event corresponding to a normalized index of 5 corresponds to propagation exactly along the field lines. Thus the interesting points in Fig. 1 involving zenith angles 10°–50° to the magnetic north correspond to larger increases in scintillation index than observed for other directions.

Table 1

Normalized index	2–2·4	2·5–2·9	3–3·4	3·5–3·9	4–4·4	4·5–4·9	5–5·4
No. of increases	13	6	3				1

It should also be pointed out here that no evidence of a change in scintillation-rate was found to be associated with propagation along the field lines.

While this evidence is somewhat fragmentary it would appear that the scintillation activity associated with propagation along the lines of the Earth's magnetic field is more pronounced than scintillation activity associated with other directions of propagation through the ionosphere. This result supports the suggestion of Rush and Colin[6] that other workers in this field should examine observations already made in order to verify the existence of this effect, which may prove to be a valuable tool in the study of the form of ionospheric irregularities.

Work on this and other aspects of the association of the scintillation of satellite signals with ionospheric irregularities is proceeding and will be reported more fully elsewhere.

This work has been sponsored by the Electronics Research Directorate of the Air Force Cambridge Research Center, Air Research and Development Command, under contract No. AF 64(500)–9.

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HYDROMAGNETICS AND STELLAR STRUCTURE

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IT is generally agreed that the microscopic processes of absorption and emission of electromagnetic radiation play a part in determining the macroscopic structure of gaseous stars. Thus the equation of hydrostatic equilibrium of a spherically symmetrical distribution of matter in radiative equilibrium is[1]:

$$\frac{d}{dR}(p_g + p_r) = -\frac{GM(R)\rho}{R^2} \qquad (1)$$

where p_g is the gas pressure, p_r the radiation pressure, G the universal constant of gravitation, $M(R)$ the mass internal to radius R, and ρ the density.

Now the emission and absorption of hydromagnetic radiation is a process usually on a macroscopic scale, and therefore will play a part in determining the structure of magnetic stars on a larger scale. Thus in determining equilibrium structure on this large scale, the radiation pressure in equation (1) should be written as the sum of electromagnetic radiation pressure and hydromagnetic radiation pressure. (In the absence of a magnetic field model, the latter is here assumed to be isotropic.) That is :

$$p_r = p_{er} + p_{hr} \qquad (2)$$

Let us consider, for example, the special case of the Sun. It is thought[2] that many features visible on the solar disk (for example, sunspots, flares, prominences) are manifestations of magnetic energy transmitted outwards from the Sun's inner regions. Further, movements and structure in the inner solar corona suggest that hydromagnetic energy may be both absorbed and generated there. Certainly solar-generated magnetic disturbance is observed at the Earth, suggesting that the Sun is luminous at hydromagnetic frequencies. Yet the Sun in all its visible parts preserves a gross constancy of structure. These things may be reconcilable through the concept of gross equilibrium under the influence of not only electromagnetic but also hydromagnetic radiation pressure. This is embodied in equations (1) and (2).

In the interior of the Sun p_{er} and p_{hr} may be of comparable importance. In the solar corona, however, p_{hr} should dominate. (Available data suggest that radiation pressures in optical and radio frequencies have negligible effects on gross movement in the corona.) Thus on the assumption of gross hydrostatic equilibrium the description of the solar corona (considered to be permeated with magnetic field (H)) under hydromagnetic radiative equilibrium is given by :

$$\frac{\mathrm{d}}{\mathrm{d}R}(p_g + p_{hr}) = -\frac{GM(R)\rho}{R^2} \qquad (3)$$

By analogy with the theory of electromagnetic radiative equilibrium :

$$\frac{\mathrm{d}p_{hr}}{\mathrm{d}R} = -\frac{K_h L(R)\rho}{4\pi V_A R^2} \qquad (4)$$

and

$$\mathrm{d}L(R) = 4\pi R^2 \rho \varepsilon_h \mathrm{d}R \qquad (5)$$

where : $L(R)$ = flux of hydromagnetic energy through concentric sphere of radius R ; K_h = coefficient of hydromagnetic opacity ; $V_A = H/\sqrt{4\pi\rho}$, the Alfvén speed ; ε_h = hydromagnetic emissivity per unit mass.

The equation (3) differs from that of Chapman[3] and Parker[4] for hydrostatic equilibrium in the solar corona which is :

$$\frac{\mathrm{d}p_g}{\mathrm{d}R} = -\frac{GM(R)\rho}{R^2} \qquad (6)$$

Because of the nature of the solution of equation (6), Parker was led to the belief that the solar corona is expanding subject to the equation :

$$\rho v \frac{\mathrm{d}v}{\mathrm{d}R} = -\frac{\mathrm{d}p_g}{\mathrm{d}R} - \frac{GM(R)\rho}{R^2} \qquad (7)$$

Equations (3) and (7) have one feature in common, namely, that they both imply a radial flux of momentum. Equation (7) implies in addition the radial movement of matter. It would seem that equation (3) offers a solution to the difficulties inherent in the solution of equation (6) without recourse to equation (7).

Quantitative argument against the use of equation (7) to describe the solar corona is as follows. First, it neglects altogether the effect of magnetic fields close to the Sun (say, within 2 solar radii) on any attempted expansion. This can only be justified if the kinetic energy density is very much greater than the magnetic energy density there. Assuming a density at the base of the corona of 2×10^8 protons per cm.^{3} and an outward velocity of 100 km./sec. the former is about 10^{-2} ergs per cm.^{3}. Such an energy density is exceeded by magnetic field of only one gauss. Larger fields than this may abound in the lower corona (cf. ref. 5). Parker considers magnetic fields only in the regions of interplanetary space where their energy density is already negligible compared to the kinetic energy density predicted by his model. Thus magnetic fields at the base of the corona may inhibit the expansion attributed to it.

Secondly, the inner corona is known to contain magnetic fields and to manifest movements which are not well correlated throughout the whole region. Let us delineate this inner region by a sphere D (of radius α) concentric with the Sun. α may be a few solar radii. Let us suppose that hydromagnetic waves are generated at the surface of D and emitted outwards into the surrounding medium which is traversed either by the solar magnetic field or an interplanetary field. The outward flow of momentum due to such emission could cause sufficient reaction on D to inhibit its expansion. Thus (if α is 2 solar radii) $2 \cdot 8 \times 10^6$ protons per cm.^{3} there[6], moving outward at 100 km./sec., would represent an outward momentum flux of about 10^{-4} erg/cm.^{3} at the surface of D. This may be inhibited by the reaction to outwardly propagated hydromagnetic waves for which the flux of momentum (F) is given by $\frac{(\Delta H)^2}{8\pi}\frac{V_A^2}{c^2}$, where ΔH is the disturbance magnetic field. Putting H = 10 gauss and ΔH = 1 gauss, $F \approx 10^{-4}$ erg/cm.^{3} on the surface of D. Of course, such hydromagnetic radiation reaction would occur in a continuous fashion all through the inner corona and may inhibit altogether the expansion of the solar corona which is inevitable on Parker's model.

In view of these criticisms, I consider it is not proved that outstreaming of coronal gas to distances of astronomical units[4] is a general condition of the Sun. However, the generation of outward-travelling hydromagnetic waves in the inner corona would seem to be an inevitable consequence of the interaction of momentum and magnetic fields there, both of which are observed to be of the utmost complexity. Moreover these waves, the wave-lengths of which may exceed the dimensions of the Earth, can cause corpuscular bombardment of the Earth and comets. A theory of solar-generated geomagnetic disturbance and auroras based on the propagation of hydromagnetic waves all the way from the Sun has been initiated earlier[7-10].

An experimental test to decide between Parker's expansion model[4] and the above hydromagnetic model of the solar corona may be provided by observation of hydrogen spectra in the night glow. On Parker's model one would expect a permanent red Doppler shift in H_α of about 10 Å., while on the hydromagnetic model one would expect only Doppler broadening of variable amounts. Shklovsky[11] reports that Prokudina's observations of H_α in the night glow showed a Doppler broadening of no more than 2 Å. However, the observation should be repeated with a design to distinguish the above two models. Of course, the contribution to hydrogen line intensities by the geocorona would have to be eliminated perhaps by a space-probe or satellite experiment.

The establishment of the usefulness of equation (3) in studying the solar corona would be strong support for the incorporation of equation (2) in the study of stellar structure in general.

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INTESTINAL ABSORPTION OF VITAMIN B_{12} AND OF A VITAMIN B_{12}-PEPTIDE COMPLEX IN NORMAL SUBJECTS AND IN PATIENTS WITH PARTIAL GASTRECTOMY

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THE partially gastrectomized patient seemed to provide adequate conditions to elaborate a test for the detection of minor impairment in intestinal vitamin B_{12} absorption, as well as to compare the absorbability of different vitamin B_{12} preparations.

Work on the behaviour of partially gastrectomized patients has led to conflicting results. Glass *et al.*[1], using surface scintillation counting of the liver after oral administration of the radioactive vitamin[2], found a decrease in radioactivity uptake by the liver in 7 out of 8 patients. More recently, Brodin *et al.*[3], using the Schilling test[4], reported a normal urinary excretion of radioactive vitamin B_{12} in 30 patients; only 4 patients had low excretion values. This would indicate the existence of a minor impairment, which I wished to demonstrate clearly. Furthermore, I compared the absorption, in normal patients as well as in partially gastrectomized patients, of crystalline vitamin B_{12} and of the vitamin B_{12}-peptide complex, isolated from a microbial fermentation.

The radioactive test had therefore to be modified to enable me to measure the amount of vitamin B_{12} absorbed by the intestine after the ingestion of a dose large enough to saturate the absorption system, for example, 10 µgm. This dose is present in a normal diet, and is certainly too small to involve a diffusion mechanism during the absorption process, which has been postulated to explain the absorption after feeding with large oral doses.

Labelled vitamin B_{12} is obtained from a culture of *Propionibacterium freudenreichii* in a medium containing cobalt-60. The labelled vitamin B_{12}-peptide complex is prepared by exchange in an acid medium with unlabelled peptide complex or by addition of the labelled vitamin B_{12} to a solution of the peptide.

1 µgm. of labelled vitamin is injected intravenously into the patient and the surface counting of the liver is recorded with a scintillation counter 2, 4, 6 days afterwards. During this time-interval, there is no appreciable excretion of radioactivity and the surface counting of the liver remains practically constant from the fourth to the thirtieth day. This measurement is used as a standard for the calculation of the amount of vitamin B_{12} or vitamin B_{12}-peptide absorbed by the intestine after oral administration. Then a known amount of vitamin B_{12} of given specific radioactivity is given by mouth and a new surface counting is made. The counts corresponding to each administration add to the previous counting.

The amount of absorbed vitamin B_{12} is given by the formula:

$$\underset{(\mu\text{gm.})}{x} = \frac{N-n}{n} \cdot \frac{R_s}{r_s} \qquad \text{(I)}$$

where N is surface counting of the liver after the oral dose, n is surface counting of the liver after intravenous injection, R_s is specific radioactivity of the injected vitamin B_{12}, r_s is specific radioactivity of the ingested vitamin B_{12}.

The percentage absorption is equal to $\frac{x}{a} \times 100$ (II),

where a is oral dose of vitamin B_{12} in µgm.

To estimate intestinal absorption, Glass[1,2] measures the liver radioactivity after an oral dose of 0·5–1 µgm. of labelled vitamin B_{12} and he expresses the results in counts/min. over the abdominal background. If he gives the amount of vitamin B_{12} absorbed, he uses formula (I), where N and n are equal to the surface counting of the liver diminished from the surface counting of the spleen, as some radioactivity is uniformly spread over the abdomen.

From Fig. 1, it is clear that the counting over the liver (A) includes the liver radioactivity plus the radioactivity of the abdomen; furthermore, the surface counting of the spleen (B) includes also some of the radioactivity present in A. The importance of this additional radioactivity can be assessed by a third measurement at C, which is symmetrical to B

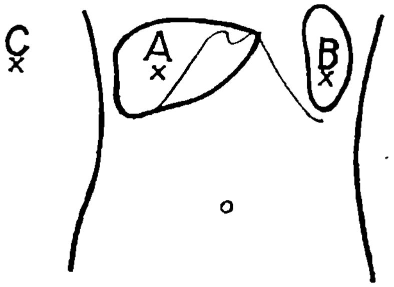

Fig. 1. Schematic representation of the abdominal projection of the liver and spleen

in respect to A and where the additional radioactivity originating from A is the same as at B. The radioactivity of the liver surface is, therefore:

$$N = N_1 - (N_2 - N_3),$$

where N_1 is radioactivity at A, N_2 is radioactivity at B, N_3 is radioactivity at C.

Experimentally, N_3 is of the order of 15 per cent of N_1.

(1) *Normal subjects.* Table 1 gives the results relative to the amount of vitamin B_{12} absorbed after an oral dose of 10 µgm.

Table 1. ABSORPTION OF VITAMIN B_{12} IN NORMAL SUBJECTS

Case	Sex	Age (yr.)	Absorption (µgm.)
1	F	32	2·5
2	M	44	4
3	M	35	5
4	M	55	4·2
			Mean 3·9

Therefore, under normal conditions, about 4 µgm. of vitamin B_{12} are absorbed after an oral dose of 10 µgm. This amount is higher than that found by Glass[5]; according to him, the amount of absorbed vitamin B_{12} is about 1 µgm. for oral doses varying from 2 to 50 µgm. My results agree with that of Reizenstein and Nyberg[6], who found 3 ± 0·9 µgm. of vitamin B_{12} retained after ingestions varying from 4 to 50 µgm.

(2) *Partially gastrectomized patients.* The results concerning 5 patients are reported in Table 2.

The intestinal absorption of vitamin B_{12} is well below normal in partially gastrectomized patients (45 per cent of the amount absorbed by the normal subject).

(3) *Absorption of a vitamin B_{12}-peptide complex.* I gave orally an amount of the labelled vitamin B_{12}-peptide complex equivalent to 10 µgm. of vitamin B_{12} to the same normal subjects as well as to the same partially gastrectomized patients. The results are shown in Table 3 in µgm. of vitamin B_{12} absorbed.

Table 2. ABSORPTION OF VITAMIN B_{12} IN PARTIALLY GASTRECTOMIZED PATIENTS

Case	Sex	Age (yr.)	Absorption (µgm.)
5	M	66	2·1
6	F	44	2·7
7	M	65	1·4
8	M	50	1·0
9	M	69	1·5
			Mean 1·8

Table 3. ABSORPTION OF THE VITAMIN B_{12}-PEPTIDE COMPLEX

Case	Normal subjects Absorption (µgm.)	Case	Partially gastrectomized patients Absorption (µgm.)
1	2·65	5	4·7
2	4	6	3·2
3	6·5	7	3·8
4	3·7	8	2·2
		9	4·7
	Mean 4·2		Mean 3·6

From Table 3, it is apparent that in the normal subject there is no difference in absorption between the vitamin B_{12}-peptide complex and vitamin B_{12} itself. In the partially gastrectomized patients, intestinal absorption of the vitamin B_{12}-peptide is twice as high as that of vitamin B_{12} and the quantity absorbed has reverted to normal values.

It is thus shown, using adequate testing conditions (10 µgm. of labelled vitamin B_{12} for the oral dose and a modified surface liver counting), that intestinal absorption of vitamin B_{12} is impaired in the partially gastrectomized patient. The vitamin B_{12}-peptide complex, isolated by Heathcote and Mooney[7], can restore completely the absorption. According to these findings, the partially gastrectomized patient is well suited to test the factors involved in vitamin B_{12} absorption.

I am indebted to Dr. Heathcote for the vitamin B_{12}-peptide complex.

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RETINAL CORRESPONDENCY AND THE HOROPTER IN ANOMALOUS CORRESPONDENCE

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VISUAL direction is of particular interest because of its remarkable delicacy and its important contribution to space perception. It rests primarily on the organization of the retinas one to the other, and both to that of the visual cortex. With continuous bifixation on a small object, it is possible to position in space a number of object-points (conventionally in a horizontal plane) such that each is seen in the same direction from the observer when viewed alternately by the eyes. This group of points constitutes one type of empirical horopter, the identical-visual-direction horopter. Study of the horopter curve has led to greater understanding of the normal organization of corresponding areas between the two retinas. In this system the centres of the two foveas correspond; moreover, one eye's temporal retinal elements (connecting neuroanatomically with cells in the ipsilateral occipital cortex) correspond with nasal retinal elements of the other eye (which connect with cortical cells in the same hemisphere).

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But the nature of anomalous retinal correspondence remains an enigma. In this form of retinal correspondence, found in many persons with strabismus ('crossed eye'), the two foveas do not correspond; instead, the fovea of one eye functionally corresponds with an extra-foveal point in the other retina. Thus, there is the curious implication that pairs of corresponding retinal points can be associated between opposite occipital hemispheres. With anomalous correspondence in esotropia, for example, certain retinal points nasal to the fovea of a deviated (right) eye and connecting with the contralateral (left) occipital cortex would be functionally corresponding with nasal retinal elements of the other eye which lead to the contralateral (right) cortex. Whether there can be correspondence between opposite occipital hemispheres depends on whether there is a horopter in anomalous retinal correspondence.

Verhoeff[1] claims there is no retinal correspondency in anomalous correspondence. However, most experimentalists intuitively believe that a horopter should be demonstrable. Although there has been an occasional paper discussing the matter, we have been unable to learn of an investigation in which the horopter has been demonstrated in anomalous correspondence. Halldén[2], who succeeded in obtaining a two-point horopter in the course of studying another feature of anomalous correspondence, noted that, " . . . in cases of anomalous correspondence investigation of the horopter is attended by very considerable difficulties". Ogle[3], who has probably had more experience with the horopter than any other researcher, remarked on the horopter in subjects with strabismus, " . . . my experience is a most frustrating one: the subjects are so indefinite as to what or how they see . . .". In spite of a lack of published reports, the indication is that investigations have been attempted, but without success.

After several years work we have been able to obtain the empirical longitudinal horopter in anomalous correspondence[4]. In this first article we wish to report only on the experimental procedure and method of analysis which we have found to be applicable in either strabismus or normal binocular vision.

The criterion of identical visual direction is used. The apparatus (Fig. 1) consists of a frame arranged in a frontoparallel plane which may be used at fixation distances of 75 cm.–6 m. An upper section contains several vertically oriented 'Plexiglass' rods which may be placed as much as 75 cm. to either side of the central fixation point; a lower section contains a cable and scale about 3 m. in length along which a mobile rod may be moved by the observer. The rods (½ in. diam., 9 in. long) are painted on one end and on the side opposite the observer. A 2·2-V., 0·25-amp. 'bullseye' bulb is placed in contact with the unpainted end of each rod. The lower mobile rod and a selected fixed rod above are alternatively illuminated at a rate adjusted for vivid perception of apparent movement (2–4 c.p.s.). Fixation is continuously maintained on a central red spot (2 mm. diam.) that is optically imaged in the middle of a ¼-in. gap between the upper and lower rods. A large sheet of 'Polaroid' filter (*HN* 34 linear polarizer) is placed with axis 45° in front of the upper rods; another sheet with axis 135° is placed before the lower rod. The head is fixed by means of a mouth-bite. 'Polaroid' filters appropriately oriented before the eyes make an illuminated upper rod visible only to the fixating (dominant) eye and the lower rod visible only to its fellow. While fixating the central red spot, the observer moves the lower rod by means of a pair of synchronous ('Selsyn') motors until the apparent movement between the alternately illuminated rods is purely vertical (that is, no lateral component in the movement is seen). The identical-visual-direction setting is greatly aided by the appearance at this point of a unique 'pouring effect' or 'piston effect'. The position of the lower rod with respect to the fixation point is read from a scale behind the rod holder.

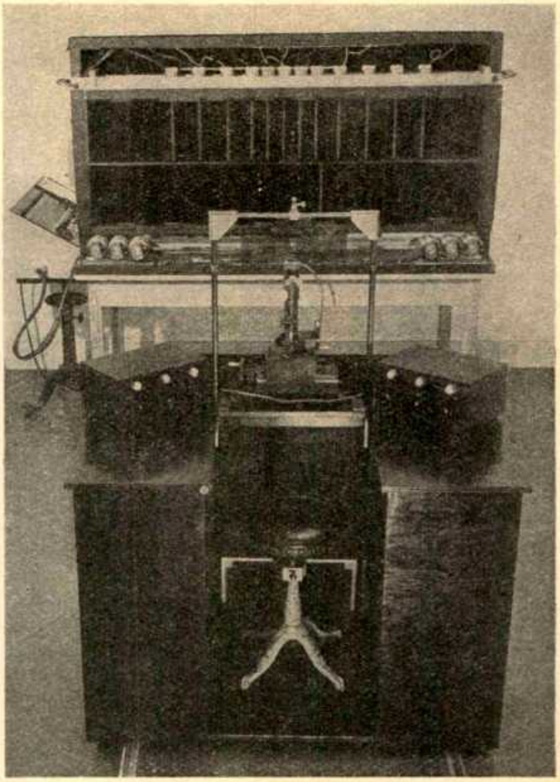
Fig. 1. The horopter apparatus

The mean setting of the lower mobile rod together with the position of the corresponding upper rod specifies a point on the horopter that lies in the visual plane. In a system of rectangular co-ordinates with the origin at the mid-point between the entrance pupil centres, x equals $a(T_L + T_R)/(2a + T_L - T_R)$ (Eq. 1), and y equals $2ab/(2a + T_L - T_R)$ (Eq. 2) where $2a$ is the interpupillary distance, b is the fixation distance, and T_L and T_R are the rod settings for the left and right eyes, respectively. The aggregate of the spatial points for which data have been obtained gives the empirical identical-visual-direction horopter.

The longitudinal angles α_L and α_R subtended respectively at the left and right eyes by the fixation point and one horopter point are obtained from the equations: $\tan\alpha_L = bT_L/(b^2 + a^2 + aT_L) = (bx - ay + ab)/(ax + by + a^2)$ (Eq. 3), and $\tan\alpha_R = bT_R/(b^2 + a^2 - aT_R) = (bx + ay - ab)/(- ax + by + a^2)$ (Eq. 4).

Ogle[5,6] has shown that for subjects with normal binocular vision the empirical horopter is satisfactorily described by a conic the skew of which, R_0, and curvature, H, at the fixation point are obtained from an analytical plot of data which are fitted to the equation $\tan\alpha_R/\tan\alpha_L = R_0 + H\tan\alpha_R$ (Eq. 5). For simplicity Ogle's mathematical model is based on the assumption that the angular separation between the fixation point and a central point on the horopter would be small compared with angle α, and that the fixation disparity responsible for this would be equal in the two eyes. We have found Ogle's model applicable in normal binocular vision, but quite inadequate for analysis of the horopter in strabismus. This is not surprising since the horopter in strabismus should be expected often to miss the fixation point by a rather large angular amount (equivalent to the 'subjective angle'). In fact, in strabismus with normal correspondence the horopter should (and our experiments indicate that it does) pass through the point of intersection of the visual axes. For this reason, as well as the fact that in strabismus the 'turning' is not equal in the two eyes but rather is confined to one eye (at a time), it became apparent to us that a new mathematical model was needed for analysis of the horopter in strabismus.

Our mathematical model includes a constant F which denotes the tangent of the angular displacement of the horopter from the fixation point, a constant M which denotes the skew of the horopter with respect to an objective fronto-parallel plane of the observer, and a constant C which denotes the curvature of the horopter where it crosses the visual axis of the fixating eye. The equation which relates these constants in a form comparable to Ogle's[6] is $U = \tan\alpha_m/\tan\alpha_f = (F/\tan\alpha_f) + M + C \tan\alpha_f$ (Eq. 6), where α_f is the longitudinal angle subtended at the fixating or dominant eye by the fixed rod and the fixation point, and α_m is the corresponding angle subtended at the other eye by the mobile rod and the fixation point. F is zero when the horopter passes through the fixation point; it is positive for eso- and negative for exo-fixation disparity when the right eye is fixating, and the reverse for left eye fixation. M is a measure of the relative magnification of the images in the two eyes. When M is greater than unity, the image is larger for the eye viewing the fixed rods; when less than unity, the image for this eye is the smaller. C represents the deviation of the empirical horopter from the theoretical curve that would result from all longitudinal angles being equal. With exact bifixation, this theoretical curve is the Vieth–Müller circle and the deviation of the empirical horopter from it is the Hering–Hillebrand deviation. The constant C is a measure of the relative asymmetry in the distribution of corresponding retinal points in the two eyes. With the right eye fixating, C is positive when the horopter has greater curvature than the theoretical curve, and negative when it has less curvature than the theoretical curve, or when it is convex toward the eyes; with left eye fixation the signs are reversed.

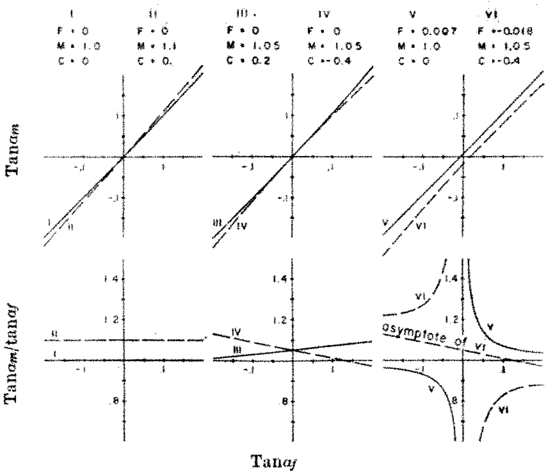

Fig. 2. Bottom, plot of $\tan\alpha_m/\tan\alpha_f = (F/\tan\alpha_f) + M + C \tan\alpha_f$ is comparable to Ogle's (refs. 5 and 6) well-known analysis of horopter data. Simple transformation gives $\tan\alpha_m = F + M \tan\alpha_f + C \tan^2\alpha_f$ (top). The constants F (displacement of horopter from fixation point), M (horopter skew), and C (horopter curvature) are the same in both plots

When F is not zero the plot of Eq. 6 is a hyperbola (Fig. 2, bottom), one asymptote being essentially the ordinate axis, the second asymptote being a straight line with the formula $U = M + C \tan\alpha_f$ (Eq. 7). This straight line becomes the entire curve (a degenerate hyperbola) when F equals zero. In either case the slope of this line gives the curvature of the horopter and M, the ordinate intercept, gives its skew. The hyperbola becomes asymptotic to the ordinate axis in quadrants I and III when F is positive, and II and IV when F is negative. The larger the absolute value of F, the greater is the eccentricity of the hyperbola.

To solve for the constants F, M and C, we have found it more convenient to use the transformed equation (Eq. 8) $\tan\alpha_m = F + M \tan\alpha_f + C \tan^2\alpha_f$. The plot of this equation is a parabola (Fig. 2, top). In this plot, F is the ordinate intercept, M is the slope, and C represents the curvature.

In our model the equation for the spatial plot of the horopter is a third-order polynomial in x and y which is difficult to use in fitting a curve to the data. It is simpler to use instead a pair of parametric equations.

The present experimental apparatus and mathematical analysis may be applied to a wider range of ocular conditions than was formerly possible, yielding comparable constants for the horopter in normal binocular vision, strabismus, and anomalous retinal correspondence. The functional relationship between the two retinas in anomalous correspondence will be reported in a subsequent communication.

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EFFECT OF CORTISONE AND RELATED COMPOUNDS ON THE BIOGENESIS OF CARTILAGE

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ADMINISTRATION of cortisone to whole animals has been shown to produce the following effects in connective tissue, including bone and cartilage: (1) Reduction in the metachromatic staining of the 'ground substances', mast cells and matrix of bone and cartilage. (2) Reduced uptake of radiosulphate ($^{35}SO_4^{2-}$) by connective tissues. These investigations, primarily associated with the names of Layton, Dziewiatkowski, Schiller and Asboe-Hansen, have been reviewed recently[1].

It must be stressed that such effects (and those described in this article) are only obtained with relatively high (pharmacological) doses of cortisone and are probably of little physiological significance. They assume greater significance when considered in relation to the undesirable side reactions attending the clinical use of cortisone, hydrocortisone and more potent chemical modifications of these hormones, as anti-inflammatory agents and for treatment of the 'collagen diseases'.

Since connective tissue is rich in mucopolysaccharide sulphates (heparin, chondroitin sulphates, keratosulphate, heparitin sulphates, etc.), it is generally concluded that cortisone depresses the overall production of sulphated polysaccharides in these tissues. This conclusion is scarcely justified by the above evidence alone, concerned as it is only with the metabolism of the sulphate groups (responsible for the metachromasia) of the connective tissue polysaccharides.

Boström and his co-workers[2,3] found that addition of cortisone depressed the incorporation of radiosulphate into slices of costal cartilage suspended in a Krebs–Ringer solution. Under these conditions and in the absence of added glucose and amino-acids, there was no significant synthesis of the polymeric components of cartilage. Boström concluded that cortisone reduced the activity of an enzyme reaction which promoted an exchange between the sulphate groups of the cartilage polysaccharides and the inorganic radiosulphate of the medium.

Other investigations[4] have indicated that cortisone has little effect on sulphate metabolism in the mucin-secreting cells of the gastro-intestinal tract. The mucins formed here contain ester sulphate, associated with the viscous polymeric constituents (mucosubstances). Once synthesized, the mucins are extruded into the gut and, being extracellular, are unlikely to enter into further enzymic exchange reactions with inorganic sulphate. By contrast the polymeric ester sulphates contained in connective tissues, though extracellular, are in more intimate association with the enzymes of cells than are components of extruded mucins. They are thus able to participate in sulphate exchange reactions which may be sensitive to cortisone.

We have now studied the effects of a number of steroid hormones and drugs which exhibit glucocorticoid and anti-inflammatory activities on the biogenesis of cartilage in tissue culture. For comparison, we also observed the effects of some non-steroid anti-flammatory agents upon this system *in vitro*.

Advantages of Tissue Culture

Under suitable conditions, cartilage may be induced to form *de novo* from chick embryonic somites[5]. Using this system, it is possible to study biogenesis of cartilage and its constituents[6]. Cartilage begins to form on the fourth day of culture, following induction. Measurements made at this time and in the subsequent three days primarily record the biogenesis of the cartilage constituents rather than any 'turn-over' of such constituents. Whole animal studies or *in vitro* studies with excised tissues from other than early embryonic sources usually record the sum of the synthetic and degradative processes. The biogenesis of a tissue component may be distinguished, however, from any subsequent turnover when it is first formed *de novo*, as in early stages of tissue cultures which are undergoing morphological and biochemical differentiation.

A further advantage of tissue culture studies is that topical effects of an applied drug or hormone may be readily observed. Such effects will not normally be the consequences of metabolism of the active principle or of mediation through other principles (for example, pituitary-adrenal axis), as they might be *in vivo*. Furthermore, any effects observed will probably be due to the actual concentration of drug or hormone present, added in known amounts to the tissue cultures. Losses and dilutions due to elimination, catabolism or storage in extracellular compartments will be reduced to an experimental minimum.

Methods

Three-day-old chick embryonic somites, when excised and placed in culture, form primarily mesenchymal tissues. If cultured in the presence of spinal cord or notochord, however, nodules of hyaline cartilage are formed[5].

Cartilage formation was therefore induced by placing somites and notochord together on lens paper floating above 1 ml. of nutrient medium (horse serum-embryo extract-balanced salt solution) which also contained $Na_2{}^{35}SO_4$ (5 microcuries)[6]. Added steroids (final concentration, $1 \cdot 5 \times 10^{-4}$ *M*) were emulsified with polyoxyethylene sorbitan monolaurate ('Tween 20'). Equivalent amounts of 'Tween' (800 μgm./ml.) were added to the control cultures. A minimum of ten cultures with each drug or hormone and ten control cultures were set up simultaneously. All cultures were maintained in a humid atmosphere at 37° C. for 6–8 days. The formed cartilage was removed and extracted successively with potassium chloride (made alkaline with potassium carbonate) and then with dilute potassium hydroxide. These extracts contain more than 90 per cent of the chondroitin sulphate present in the cartilage. Little or no keratosulphate was synthesized by this newly formed cartilage[6]. Sulphate ions were

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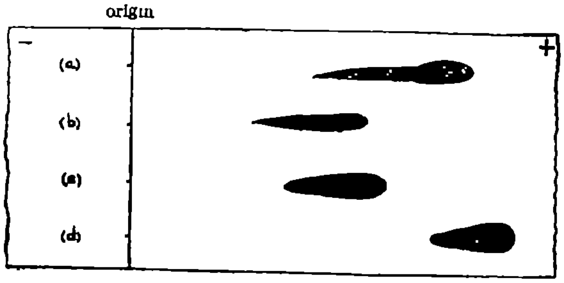

Fig. 1. Paper ionophoresis of metachromatic polysaccharides: (*a*) from normal cultures; (*b*) from cultures grown in the presence of hydrocortisone (10^{-4} *M*); (*c*) chondroitin prepared by chemical desulphation of chondroitin sulphate *A*. (*d*) chondroitin sulphate(s) extracted from bovine trachea. (Ionophoresis at 23° on Whatman 3 *MM* paper 0·2 *M* sodium borate, *p*H 8·6, 4 hr., with potential gradient of 12 V./cm. Metachromasia was detected by immersion in 0·15 per cent (w/v) thionine in 65 per cent (v/v) ethanol, acidified with 0·1 per cent (v/v) acetic acid. Excess stain was removed with 95 per cent ethanol)

removed by prolonged dialysis of the extracts, after the additions of non-radioactive sodium sulphate. Aliquots were dried, and incorporated sulphur-35 measured by direct counting. The uronic acid content of these dialysed extracts was measured colorimetrically using the carbazole–sulphuric acid reactions[7]. Full experimental details have been given elsewhere[6].

Results with Cortisone and Hydrocortisone

Both these hormones severely depressed the growth of cartilage as observed microscopically. The uptake of radiosulphate into the chondroitin sulphate fraction was also much reduced. However, the amount of non-dialysable uronic acid found in extracts from cartilage grown in the presence of these hormones was not significantly less than that present in extracts of the control cultures (Table 1).

When extracts from the cortisone and hydrocortisone-treated cultures were examined by paper ionophoresis (Fig. 1) it was found that the principal metachromatic component had a lower ionophoretic mobility than mixtures of chondroitin sulphates *A* and *C* extracted from animal cartilage (bovine nasal septa, trachea, chicken costal cartilage, elasmobranch endoskeleton, etc.), but corresponded in mobility to a chemically desulphated[8] preparation of chondroitin sulphate *A* (containing about 2 per cent residual ester sulphate). Extracts from control cultures contained as their principal metachromatic components, chondroitin sulphate(s) with ionophoretic mobilities almost identical with authentic specimens from animal cartilage (15 per cent ester sulphate).

These analytical findings suggest that cortisone and hydrocortisone do not primarily affect the biosynthesis of the polysaccharide portion (chondroitin) of the chondroitin sulphate (containing glucuronic acid and N-acetylgalactosamine) but that they do influence its subsequent sulphation.

Table 1. EFFECT OF SOME HORMONES AND DRUGS ON (*a*) THE INCORPORATION OF RADIOSULPHATE INTO CHONDROITIN SULPHATES (*s*) AND ON (*b*) THE NON-DIALYSABLE URONIC ACID FRACTION EXTRACTED FROM CARTILAGE FORMED *de novo* IN TISSUE CULTURE

Compound (final conc. added) 1·5 × 10^{-4} *M*	No. of cultures	Uronic acid content	$^{35}SO_4^{2-}$ incorporated
None	2 × 10	100*	100*
Hydrocortisone	5 × 10	100	27
	20	120	19
Cortisone	5 × 10	100	28
Progesterone	2 × 10	100	100
Testosterone	10	100	100
Deoxycorticosterone	20		100
Prednisone	10		26
Prednisolone	3 × 10	80	0

* Controls were assigned a value of 100

The deoxyribonucleic acid contents of both the cultures treated with hydrocortisone (or prednisone) and of the control cultures increased by only 30 per cent during the period required for cartilage-formation. The effects of hydrocortisone cannot therefore be explained simply as an inhibition of cell multiplication and loss of viability during this period. This was also directly demonstrated by experiments in which somites and notochord were exposed to hydrocortisone for various intervals less than the four days required for the appearance of normal cartilage in control cultures. When the tissues were afterwards transferred to hydrocortisone-free growth medium, they recovered their capacity to make normal cartilage, took up radiosulphate and formed metachromatic material with the same ionophoretic mobility as chondroitin sulphate.

The following considerations would seem to eliminate one further explanation of our analytical results, namely, that the steroid hormones are activating an enzyme system (chondrosulphatase) which removes ester sulphate from initially formed chondroitin sulphates. Though sought, such a chondrosulphatase has never been described in mammals. Chondrosulphatases have only been reported to be present in some bacteria and certain molluscs[9,10]. When normal cultures were grown in the radiosulphate medium for 5–8 days and afterwards transferred to a medium containing no $^{35}SO_4^{2-}$, the rate of loss of incorporated radioactivity due to subsequent turnover and exchange of the ester sulphate groups was not affected by the presence of cortisone or hydrocortisone in this second medium. The result seems to exclude an activation of any chondrosulphatase enzymes present in the cartilage cultures by these steroids.

Adrenocorticotrophic hormone had no effect on cartilage formation in this system, as might be expected if it is the adrenal corticoids themselves which are pharmacologically active. Other steroid hormones (deoxycorticosterone, progesterone, testosterone, corticosterone) were without measurable effect on chondrogenesis.

Results with Some Synthetic Glucocorticoids

The following compounds were tested: prednisone (Δ^1-cortisone) and prednisolone (Δ^1-hydrocortisone) (Merck, Sharp and Dohme); the 6α-methyl derivative of cortisone, hydrocortisone, prednisone and prednisolone (Upjohn Co.); the 9α-fluoro and 9α-fluoro, 16α-hydroxy derivatives of hydrocortisone and prednisolone (E. R. Squibb and Sons).

All these, with the exception of 6α-methylprednisolone (Medrol), depressed cartilage and chondroitin sulphate synthesis at concentrations of 1·5 × 10^{-4} *M*. They were nearly all as potent as hydrocortisone. In some instances, for example, with prednisolone, 16α-hydroxy-9α-fluorohydrocortisone, they were more active as inhibitors of chondrogenesis. Their inhibitory effects generally paralleled their relative anti-inflammatory properties[11]. Insufficient determinations were made with each drug to assess accurately their relative potencies.

As with cortisone or hydrocortisone, the sulphation rather than the biosynthesis of (unsulphated) chondroitin was primarily inhibited.

Results with some Non-steroid Anti-inflammatory Agents

Potassium salicylate at high concentrations ($1 \cdot 5 \times 10^{-3}$ *M*) greatly depressed chondrogenesis and was remarkably specific in its action. Its close chemical analogues, potassium benzoate and potassium thiosalicylate, were quite without effect at this concentration. Boström and Månsson[2] have previously reported the cortisone-like effect of salicylate on the sulphate exchange reaction in cartilage slices *in vitro*. Further analyses indicated that, as with cortisone, sulphation but not the biosynthesis of the chondroitin was chiefly affected.

Phenylbutazone and cinchophene at concentrations of 3×10^{-4} *M* had no detectable action on chondrogenesis *in vitro*.

Discussion and Conclusions

Cortisone, hydrocortisone and their chemical analogues apparently inhibit the formation of acid mucopolysaccharides in connective tissue, primarily by inhibiting the sulphation of the pre-formed polysaccharide. Hvidberg and Jensen[12] have recently shown that cortisone administration leads to loss of metachromasia and water content in the polysaccharide fraction of mouse skin. After cortisone treatment the average molecular weight of this mixture of polysaccharides was 20 per cent lower than in the controls, but the amino-sugar content was unchanged. These findings suggest that cortisone likewise inhibits sulphation, but not the synthesis, of the polysaccharide portion of polysaccharides *in vivo*. Failure to form normal amounts of the acidic macromolecule (for example, chondriotin sulphate) would in turn affect deposition and orientation of the basic collagen-like proteins of connective tissue. Other properties depending on the presence and maintenance of the acidic sulphate groups on a structural component (serving as natural cation exchanges) such as retention, deposition or movement of ions and water, would also be grossly influenced by cortisone treatment.

Recent work on the biosynthesis of chondroitin sulphates[13–15] indicates that sulphation of chondroitin only occurs at a very late stage in the overall biosynthetic sequence. Incorporation of inorganic sulphate into, and subsequent polymerization of, hexose sulphates seems to be excluded as a major biosynthetic process. Sulphate ions are activated for metabolism by reaction with adenosine triphosphate to form adenosine-3′-phosphate-5′-phosphosulphate (PAPS)[15]. PAPS is formed by extracts of costal cartilage[17], liver[16,18] and hen oviduct[14], which also contain enzymes which will transfer the sulphate group from PAPS to chondroitin and partially sulphated chondroitin[13–15]. We have failed to find any direct inhibition by hydrocortisone or prednisolone ($10^{-4}M$) of PAPS formation or of the transfer of sulphate from PAPS to phenolic acceptors by soluble enzymes from rat liver[18], intestinal mucosa or kidney[19].

The one tissue where cortisone can usefully be applied clinically to suppress inflammation, the eye, normally contains in the cornea significant amounts of unsulphated chondroitin (25 per cent of the total polysaccharides) as a structural component[20]. Is it mere coincidence that in this tissue, where inhibition of sulphation would have the least consequence, cortisone has such a beneficial effect in arresting inflammation and so little undesirable side effect? Here, there would be in essence, anti-inflammatory activity without anti-sulphation activity. This desirable end is found otherwise only with certain non-steroid drugs, for example, phenylbutazone.

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INCREASED DIHYDROFOLATE REDUCTASE ACTIVITY AS A POSSIBLE BASIS OF DRUG RESISTANCE IN LEUKÆMIA

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LEUKÆMIC cells initially sensitive to the action of folic acid analogues develop resistance to inhibition by these drugs[1]. It has been suggested that the decreased efficacy of the antifolates may be due in part to the increased capacity of resistant cells to reduce folic acid compounds to the coenzymatically active tetrahydro-forms[2–6]. This theory is attractive since the antifolates are known to be extremely effective inhibitors of the enzyme system which catalyses the reduction of folic acid to tetrahydrofolic acid[7–10].

In the present investigation dihydrofolate reductase activity has been estimated in tissues of mice with

Table 1. SUB-LINES OF MOUSE LYMPHOID LEUKÆMIA *L*1210 USED IN THIS WORK

All sub-lines were carried in hybrid male mice (*BALB/c An* × *DBA/2J*) F_1. (Referred to commonly as *CDBA*)

Sub-line	Developed by	Generations untreated	Resistance to		Ref.
			Amethopterin	Dichloro-amethopterin	
M46R	Amethopterin treatment of *L*1210	81	complete	moderate	11, 12
*M*66–3R	Dichloro-amethopterin treatment of *L*1210	17	marked	marked	12, 13
C82A	Dichloro-amethopterin treatment of *M46R*	8	complete	complete	13
8-**Aza-guanine resistant**	8-Aza-guanine treatment of *L*1210	58	none	none	14

antifolate-sensitive and antifolate-resistant sub-lines of mouse lymphoid leukæmia *L*1210. Marked increases of enzyme activity have been found in resistant cells grown for many generations in the absence of the antifolates. The implications of high enzyme-levels are discussed not only in relation to the emergence of resistant cells but also to possible utilization of this activity to achieve lethal synthesis with new kinds of folic acid antagonists.

During investigations on the influence of chemotherapeutic agents against systemic lymphoid leukæmia (*L*1210) in mice, various antifolate-resistant sublines were developed. A summary of information on these sublines is given in Table 1. In addition to the antifolate-resistant sub-lines, an 8-azaguanine variant of leukæmia *L*1210 was also studied. It had been produced by treatment with 8-azaguanine over a number of generations[14]. This variant is cross-resistant to 6-mercaptopurine and shows increased sensitivity to amethopterin[14].

The tissues used were obtained from normal 12-week-old *CDBA* male mice and from mice of the same cross implanted with various lines of antifolate-sensitive and antifolate-resistant lymphoid leukæmia. The tumour tissue was solid tumour which grew at the site of implantation. 8–11 days after inoculation the mice were killed, and the tissues were removed and quick-frozen in beakers submerged in solid carbon dioxide and acetone. One part of frozen tissue (usually the combined material from several animals, Table 2) was homogenized in a Waring Blendor with ten parts of acetone at −20°, collected on a paper filter, washed three times with cold acetone and finally dried at room temperature *in vacuo*. The powders were stored *in vacuo* at 2–4°. Extracts were prepared by mixing one part of acetone powder with ten parts of 0·05 *M* potassium phosphate buffer *p*H 7·5–0·01 *M* 2-mercaptoethanol for 15 min. at 0° and then centrifuging at 10,000 r.p.m. at 4° for 45 min. The supernatant fluid was used for the enzyme assays.

The enzymatic oxidation of reduced triphosphopyridine nucleotide by dihydrofolate was determined by measuring the decrease in absorbance at 340 mμ over a 5-min. period[15]. The complete incubation mixture consisted of 3·0 ml. of 0·05 *M* potassium phosphate buffer *p*H 7·5–0·01 *M* mercaptoethanol, 0·1 μmole of dihydrofolic acid (prepared by reduction of folic acid with sodium dithionite[16]), 0·2 μmole of reduced triphosphopyridine nucleotide and 50 μl. of enzyme extract. The number of millimicromoles of dihydrofolate reduced was calculated from the decrease of absorbance at $\lambda = 340$ mμ on the basis of $\Delta\varepsilon = 11{,}270$ due to the combined decrease of $\Delta\varepsilon = 6{,}220$ (reduced triphosphopyridine nucleotide oxidized to triphosphopyridine nucleotide) plus $\Delta\varepsilon = 5{,}050$ (dihydrofolate reduced to tetrahydrofolate). Protein content was determined by the method of Lowry *et al.*[17]. Each value was corrected for a minor change in absorbance which occurred in the absence of dihydrofolic acid.

The enzyme activity data are summarized in Table 3. A comparison of dihydrofolate reductase activities in the solid tumours which had grown at the site of implantation of leukæmic cells reveals the following: *M46R* (sub-line of *L*1210 which had developed resistance to amethopterin) had four times the activity of the sensitive *L*1210. *M66–3R* (sub-line of *L*1210 which had developed resistance to dichloroamethopterin) showed no increase in activity over that of the sensitive *L*1210. However, *C82A* (sub-line of *L*1210 made doubly resistant to amethopterin and dichloroamethopterin) had more than 29 times the activity of the original sensitive line. Thus when two closely related folic acid antagonists were used in sequence to develop a sub-line of leukæmia which was doubly resistant to both drugs, leukæmic cells emerged with very high levels of dihydrofolate reductase activity. Enzyme induction was probably not involved since high levels of enzyme were maintained in resistant cells grown for many generations in the absence of antifolate.

The enzyme activities of spleens of leukæmic animals were essentially the same as those obtained with the solid tumours. On invasion by leukæmic cells the spleen increased from a normal average weight of 0·1 gm. to 0·5 gm. Thus approximately 80 per cent of the spleen may have consisted of leukæmic cells. The assumption here would be that the leukæmic process had not caused in addition the entrance or proliferation of normal cells. After invasion by leukæmic cells the spleen, which normally

Table 2

Source of tissues	Average wet weight (gm.)			Animals per assay			Number of assays		
	Liver	Spleen	Tumour	Liver	Spleen	Tumour	Liver	Spleen	Tumour
Normal	1·67	0·10	—	7	6	—	7	8	—
*L*1210	2·41	0·50	0·20	6	7	7	7	6	5
M46R	2·44	0·52	0·27	6	7	8	6	5	4
M66–3R	1·84	0·58	0·26	5	5	8	4	4	3
C82A	2·50	0·50	0·30	3	3	8	5	4	4
Azaguanine resistant	2·50	0·75	0·60	4	4	4	2	2	2

Table 3. DIHYDROFOLATE REDUCTASE ACTIVITIES

Source of tissues	mμmol. of dihydrofolate reduced per mgm. of protein per hr. at 28° (mean ± *S.E.*)		
	Liver	Spleen	Tumour
Normal	51 ± 6	11*	—
*L*1210	60 ± 8	59 ± 7	53 ± 10
*M*46*R*	113 ± 10	222 ± 26	225 ± 41
*M*66–3*R*	52 ± 4	55 ± 11	53 ± 5
*C*82*A*	413 ± 88	1,340 ± 155	1,535 ± 105
Azaguanine resistant	61†	68†	75†

* In five assays the activity was almost zero.
† Average of two assays.

had very little if any dihydrofolate reductase, exhibited markedly increased enzyme activity of the same order as that observed for tumour.

The infiltration of the liver by leukæmic cells, although not as extensive as in the spleen, was also accompanied by an increase of dihydrofolate reductase activity. Again the pattern of activities of the various sub-lines paralleled the tumour activities. If it is assumed that the increased weight of the livers from leukæmic mice (Table 2) is caused by infiltration of leukæmic cells having the same enzyme activity as the cells of the solid tumour, then it is possible to calculate the approximate enzyme activity which can be expected in various samples of leukæmic liver. For *M*46*R* liver the predicted value obtained in this manner was 106 as compared with an actual value of 113 (Table 3). For *C*82*A* liver the predicted and observed values were 544 and 413 respectively.

Attempts to explain the phenomenon of resistance should be tempered with the realization that knowledge of the biochemical basis of resistance gained by study of one type of cell may not be applicable to other kinds of cells. It has been suggested that the major factor in the development of resistant *S. faecalis* is the reduction in the permeability of the bacterial cell to the antifolates[18]. The occurrence of such a change in leukæmic cells has not been definitively established. Aronow has attributed the resistance of mouse fibroblast cells grown in tissue culture to possible changes in enzymes concerned with tetrahydrofolic acid utilization[19].

Table 4. INHIBITORY EFFECTS OF THREE ANTIFOLATES ON DIHYDROFOLATE REDUCTASE ACTIVITIES OF SENSITIVE AND RESISTANT LEUKÆMIAS

Source of enzyme	Uninhibited enzyme	Enzyme + aminopterin $1{\cdot}0 \times 10^{-8}$ *M*	Enzyme + amethopterin $0{\cdot}5 \times 10^{-8}$ *M*	Enzyme + dichloroamethopterin $0{\cdot}25 \times 10^{-8}$ *M*
Normal liver	38	12	18	15
*L*1210 spleen	38	10	11	11
Azaguanine-resistant spleen	43	11	11	12
Dichloroamethopterin-resistant spleen, *M*66–3*R*	37	12	12	11
Amethopterin-resistant spleen, *M*46*R*	106	65	58	83
Amethopterin and dichloroamethopterin-resistant spleen, *C*82*A*	800	700	650	645

Twenty μl. of enzyme extract and 2 16 μl. of inhibitor in 2 per cent sodium carbonate (final concentration of antifolate is shown in the table) were pipetted into 1·0 ml. of 0·05 *M* potassium phosphate buffer, *p*H 7·5, 0·01 *M* 2-mercaptoethanol and incubated at 34° for 3 min. Then 0·07 μmole of reduced triphosphopyridine nucleotide and 0·02 μmole of dihydrofolic acid were added. All activities are expressed as decrease of absorbancy at 340 mμ in 5 min. All values are corrected for optical density changes which occur in absence of dihydrofolic acid and have been multiplied by 1,000 for greater ease of comparison. The 5-min. values for *C*82*A* enzyme are based on optical density changes obtained during the first minute of incubation because the high activity leads to rapid depletion of the substrate

The experimental approach itself could conceivably influence the way a cell becomes resistant to a drug. As emphasized by Nichol and Welch[18], whereas bacterial cells or neoplastic cells grown in tissue culture can be exposed to high concentrations of an antagonist in the test-tube, leukæmic cells in mice can be exposed only to a concentration of drug that can be tolerated by other tissues of the host.

If the inhibition of leukæmic cell growth by amethopterin is due mainly to the inhibition of dihydrofolate reductase, the simplest explanation of resistance would be either an increase of the enzyme or a decrease in permeability to the drug or both. So long as drug-levels *in vivo* are maintained at low levels and cell permeability is not limiting, leukæmic cells with high dihydrofolate reductase activity should have a better chance to survive than cells with low dihydrofolate reductase activity. It is easy to demonstrate with cell-free extracts *in vitro*, where permeability does not play a part, how high levels of dihydrofolate reductase could help to explain resistance. For example, despite inhibition by amethopterin at a concentration of $0{\cdot}5 \times 10^{-8}$ *M*, there was more dihydrofolate reductase activity left in the inhibited enzyme preparation from resistant leukæmic cells than in the uninhibited extracts of sensitive *L*1210 cells (Table 4). This difference cannot be attributed to a change in affinity of drug with enzyme since preliminary kinetic studies with enzyme extracts from sensitive *L*1210 and resistant *C*82*A* spleens indicate that the K_I values with amethopterin for both enzymes are approximately the same, 5×10^{-11} *M*. Recently, Hakala *et al.*[20] and Fischer[21] have reported that amethopterin-resistant cells grown in tissue culture have markedly increased levels of folic acid reductase with no change in drug-enzyme affinity.

If the main site of amethopterin-binding in tissue is the enzyme it inhibits[20–24], and drug ingress is not limiting, then a high extracellular concentration of the drug (relative to the very low dissociation constant of enzyme-inhibitor complex) should result in cells with completely inhibited enzyme. One would expect on this basis alone that leukæmic cells with high enzyme-levels such as in *C*82*A* would sweep up the drug to a point of complete inhibition. In this situation cells with a genetic capacity to produce more enzyme would not necessarily be favoured during a period of selection of drug-resistant cells. Amethopterin at a daily optimal dose-level of 1·0 mgm. per kgm. effectively prolongs the life-span of mice with advanced leukæmia[25]. This dose is equivalent to a calculated peak-level (assuming uniform distribution) in the tissues of 2×10^{-6} *M*, a concentration which should be more than enough to inhibit completely dihydrofolate reductase activity in extracts.

The data in Table 5 show that within 4 hr. after injection of various antifolates into leukæmic mice, the dihydrofolate reductase-levels of the doubly resistant sub-line *C*82*A* tissue were much above the enzyme activity of sensitive cells. During this period the drugs had obviously been absorbed from the site

Table 5. *In vivo* EFFECTS OF THREE ANTIFOLATES ON DIHYDROFOLATE REDUCTASE ACTIVITIES OF SENSITIVE AND RESISTANT LEUKÆMIAS

Source of tissues	mμmol. of dihydrofolate reduced per mgm. of protein per hr. at 28°			
	No drug*	Amino-pterin 0·1 mgm./kgm.	Ametho-pterin 1·0 mgm./kgm.	Dichloro-ametho-pterin 100 mgm./kgm.
Azaguanine-resistant, antifolate-sensitive leukæmia				
Liver	61	1·2	1·1	0
Spleen	68	1·0	0·5	1·8
Tumour	75	1·4	0·4	0
Antifolate-resistant leukæmia, *C*82*A*				
Liver	418	301	259	36
Spleen	1,340	723	862	121
Tumour	1,535	1,720	1,460	580

* Data from Table 3 for comparison.

Antifolates were dissolved in 2 per cent sodium bicarbonate and administered subcutaneously in the axillary region. Four hr. later the mice were killed, tissues removed and assayed as described elsewhere in this article.

of subcutaneous injection, since in a control experiment the enzyme activity was almost abolished in tissues of mice implanted with the antifolate-sensitive azaguanine-resistant variant of *L*1210.

It is concluded that although increased dihydrofolate reductase activity may be an important factor in the development of resistance in the mouse leukæmia sub-lines, equally as important may be a limitation on the entry of the antifolates into the leukæmic cell. That increased dihydrofolate reductase activity cannot be the sole basis of resistance is apparent from our work with the sub-line *M*66-3*R* which had become resistant to dichloroamethopterin without a change of enzyme-level (Table 3). This, however, may be a unique situation associated with certain puzzling properties of the interesting dichloro-derivative.

Experiments with mice show that the optimal therapeutic dose of dichloroamethopterin is approximately 100 times that of amethopterin[26]; yet on the basis of our enzyme work, dichloroamethopterin is approximately 3–4 times as potent a dihydrofolate reductase inhibitor as aminopterin (Table 4). This may be due to a permeability barrier in the leukæmic cell wall to dichloroamethopterin. It is also of interest to point out that cells that had become resistant to amethopterin still showed some sensitivity to dichloroamethopterin. Another example of the uniqueness of dichloroamethopterin is that it produced apparent 'cures' in the mouse leukæmia system whereas aminopterin and amethopterin merely prolong the life-span of leukæmic mice[26]. 'Cured' mice develop an immunity to re-inoculation with fresh leukæmic material[12]. Dichloroamethopterin, despite its similarities to amethopterin, that is, inhibition of dihydrofolate reductase and reversal of its actions in animals with citrovorum factor[26], may owe its therapeutic efficacy to some other locus of action, perhaps the blocking of folic acid entry into cells.

If the resistance in human leukæmia can be shown to be comparable to that found in our work on mouse leukæmia, that is, a marked increase of dihydrofolate reductase occurring in leukæmic cells that have developed resistance to antifolates, then analogues of folic acid which act quite differently from aminopterin or amethopterin should be synthesized. The analogue should have the following properties: rather than inhibiting dihydrofolate reductase, it would act as a substrate for the enzyme. In its oxidized form it should be non-toxic and need not be effective against leukæmic cells that are sensitive to the action of amethopterin. The new agent after reduction should then be capable of inhibiting various reactions involving tetrahydrofolic acid. For example one might seek for a tetrahydrofolate analogue which inhibits the biosynthesis of thymidylic acid, methionine or purines. According to this reasoning, those cells which have become resistant due to an increased dihydrofolate reductase activity could produce more lethal synthesis of a reduced pteridine which could then have the unique property of inhibiting various tetrahydrofolate-requiring metabolic reactions. The new antifolate would not be used until resistance developed and only in situations where the resistance was due to higher levels of dihydrofolate reductase in leukæmic cells. There are at least two examples where resistance appears to be due to the inability of a cell to carry out lethal synthesis, namely, the conversion of 6-mercaptopurine (or 8-azaguanine)[27] and the conversion of 5-fluorouracil[28] to the corresponding nucleotides. The situation envisaged in this article is quite the opposite; resistance would lead to lethal synthesis if the proper antifolate were used only after resistance had occurred.

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LETTERS TO THE EDITORS

SPACE SCIENCE

Existence of Net Electric Charges on Stars

V. A. Bailey[1] has urged that a variety of geophysical and astrophysical phenomena can be explained by a net charge on the Sun of $-1 5 \times 10^{28}$ e.s.u., admitting that this proposal is at variance with some of the "most cherished notions"[2] of theoretical astrophysicists. Attention may therefore be directed to certain deductions from Bailey's hypothesis that are in conflict with unambiguous evidence from observation as well as with what is generally regarded as sound theory.

(1) Bailey suggests two explanations of how the solar charge can be produced and maintained. The first derives from the assumption that neutrinos carry a positive charge in the range between 10^{-15} and 10^{-18} times the electron charge. Now, since the conductivity of the solar material is of the order of 10^{16}–10^{17} e.s.u. (ref. 3), the field created by the leaking of positive charges from the solar core would tend to push electrons off the solar surface at such a rate that there would be no net charge left on the Sun. Accordingly, electrons would have to drift outward, and whether a sufficiently high rate of escape materializes, or fails to, depends on the drift velocity required to compensate the postulated rate of charge production entailed by the nuclear reactions. From Bailey's figures we conclude that less than 1 electron per cm.^{2} and sec. must leave the solar surface. This rate of escape corresponds to a mean drift velocity of free electrons, in the photosphere, for example, of much less than 1 cm./sec., which is a negligible fraction of the average thermal velocity. By the same argument, it follows that this outward current would not stop in the solar corona, or even in interplanetary space. We do not carry this argument forward beyond the confines of the planetary system, since there cannot result a net charge within, say, the Earth's orbit.

(2) Having concluded that the charge production must be so fast that it cannot be compensated by currents, one might fall back on Bailey's alternative explanation and postulate the existence of a poly-dimensional space, such that charge conservation need not hold exactly in our four-dimensional world. The steady-state charge would be determined essentially by the local value of the conductivity and might follow closely the mass distribution. Then, of course, the atoms of the photosphere are moving in an electric field corresponding to a point charge of $-1{\cdot}5 \times 10^{28}$ e.s.u. at the centre of the Sun. This field ought to produce an excessive Stark effect. A simple calculation with well-known formulæ[4] shows that, for example, the numerous Stark components of the H_β-line would spread over 1000 Å., or so. The observed line width is of the order of 1 Å. In particular, and this is the crucial point, in the case of H_β no undisplaced component should be found at 4861 Å. This implication of Bailey's hypothesis is simply not borne out by the solar observations. By the same argument one can rule out electric fields in the chromosphere (flash spectrum) and the corona (ultra-violet emission lines). Hence, Bailey's second mechanism is incompatible with all spectroscopic evidence.

(3) Bailey's hypothesis predicts the wrong polarity for the general magnetic field of the Sun at the present epoch. Even if one agrees with Bailey in regarding the recent reversal of the solar field as the outcome of some intervening process, one may well question the appropriateness of calling this reversal a "secondary effect"[2], inasmuch as the primary effect was predicted with the wrong sign. Neither is it helpful to appeal, in support of his hypothesis, to the fact that for five stars the ratio of magnetic moment to angular momentum was supposed to be constant. It is well known that the early data on magnetic stars referred to are superseded[5]. Rather it has become obvious that the main feature of stellar magnetism is its enormous variability, and there is now little point in appealing to a mechanism which can produce only a constant field.

(4) Bailey's model could be subjected to test by terrestrial experiments. The ionosphere, according to Bailey, shields the Earth from the solar electric field, but not from its magnetic effects. Consequently, if magnetically polarizable particles are accelerated, to nearly the velocity of light, in a direction perpendicular to the radius vector Sun–Earth, in the reference frame of such particles there would exist a magnetic field of about 100 gauss, and it should be possible to measure the resulting polarization. Also, observers travelling by jet planes past the same point in opposite directions, perpendicular to the radius vector Sun–Earth, would record values for the local field differing by 20γ, provided that the direction of flight was so chosen as to make the excess field parallel to the local terrestrial field.

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The following comments may be made on the communication by Oster and Philip concerning my article in *Nature*[1] and note in the *Journal of Atmospheric and Terrestrial Physics*[2].

Their introductory paragraph states: "Attention may therefore be directed to certain deductions from Bailey's hypothesis that are in conflict with unambiguous evidence from observation as well as with what is generally regarded as sound theory".

This claim cannot relate to their sections 1 and 2, for these refer only to two subsidiary hypotheses on the origin of the solar charge rather than to the main hypothesis, namely, its existence. At most, the arguments in these sections can lead only to the conclusion that the corresponding hypotheses of origin, H_1 and H_2, are untenable and that the origin of the solar charge remains to be discovered. Thus, so far as these two sections are concerned, the main hypothesis, that the Sun carries a large negative charge, remains intact.

Also, the argument in their section 2 which rules out the existence of strong electric fields below the coronal regions in which ultra-violet lines occur could serve as well to locate the solar charge mainly above these regions as to exclude the hypothesis H_2. This location is consistent with the conclusions given in the addendum to a paper published elsewhere[3].

The assertion made in their section 3, that "Bailey's hypothesis predicts the wrong polarity for the general magnetic field of the Sun at the present epoch", is itself wrong, for the Sun rotates like the Earth from west to east, and so its negative charge would impart to it a magnetic moment which is directed from north to south, with the result that the polar fields of the Sun would have the same directions as those observed by Hale and Langer, and, since 1958, by Harold D. Babcock[4]. This assertion by Oster and Philip could be right only if their phrase "at the present epoch" refers to some period later than the date of Babcock's report.

Their criticism, in section 3, of the original appeal to Blackett's comparison of the mean of five stars with the Sun does not affect the hypothesis on the solar charge; it merely throws doubt on Blackett's conclusion and so removes from consideration the last of the five different astronomical phenomena which the main hypothesis helped to explain.

The experimental tests suggested in their section 4 may be worth making provided that it is borne in mind that the medium surrounding the Earth may act as a partial magnetic screen. But, in accordance with the more recent revised estimate[3] of the solar charge as being about -3×10^{37} e.s.u., their estimate of the polarizing field should be reduced from 100 gauss to about 20 gauss or less and their estimate of the difference between the field intensities recorded by observers in oppositely travelling jet planes should be reduced from 20γ to 4γ or less.

Other experimental tests have been suggested earlier[1,3,5]. The launching, on March 11 last, of the solar artificial satellite *Pioneer* V has now (inadvertently) provided three of these tests. For the findings of the magnetometer of this satellite between March 11 and April 30 have now been published and so may be compared with the predictions, on apparent interplanetary fields, to which one is led by considering alone the hypothesis on the solar charge.

Such a comparison is made in the following communication. There it is shown that from the magnetic findings of *Pioneer* V during the first fifty days of its motion and from the simultaneous magnetic records made at Fort Belvoir (near Washington, D.C.) it is possible to arrive at the following conclusions concerning the quiet-time field vector $\mathbf{B}_q$ and the storm-time field vectors $\mathbf{B}_s$ recorded by the magnetometer in *Pioneer* V: (1) $\mathbf{B}_q$ was perpendicular to the ecliptic; (2) $\mathbf{B}_q$ was very probably directed from north to south; (3) the magnitude of $\mathbf{B}_q$ was about 2·5 gammas; (4) the storm fields $\mathbf{B}_s$ were very probably perpendicular to the ecliptic and directed from north to south, while the peak value of these fields was about 50γ.

The conclusions (1) and (2) agree exactly with the direction and sense of the magnetic field $\mathbf{H}_r$ induced by the motion of a negatively charged Sun relative to an inertial frame attached to the satellite. The conclusion (3) is within a factor of about 50 of the value 130γ for $\mathbf{H}_r$ which is yielded by the revised estimate -3×10^{37} e.s.u. for the solar charge; this suggests that *Pioneer* V may have been surrounded by a diamagnetic cloud of ionized hydrogen. The conclusion (4) promises to be significant in regard to theories of geomagnetic storms.

These conclusions and the discussion in the previous paragraph constitute evidence in favour of the solar charge hypothesis that is at least as cogent as most evidence against it, which is based on long-current views about the physical conditions in extra-terrestrial space or in the Sun. For nearly all such views are the result of combining direct observation from the Earth's surface with current extrapolations of physical theories to large and remote astronomical bodies or regions and it is well known that this practice has more than once led to erroneous conclusions.

With regard to any other astrophysical evidence which may be adduced for or against the solar charge hypothesis, it is essential to apply the present general principle of theoretical physics (which may be extended to include astrophysics and cosmology) that the sole purpose of this subject is "to calculate results that can be compared with observation" (Dirac).

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Nov. 24.

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Apparent Steady Component of the Interplanetary Magnetic Field

IN a recent communication, P. J. Coleman, jun., C. P. Sonnett, D. L. Judge and E. J. Smith[1] gave a preliminary report on findings provided by the magnetometer carried by the space probe *Pioneer* V during the first 50 days of operation. For brevity, this publication will be referred to as ref. 1.

In a later publication, P. J. Coleman, jun., Leverett Davis and C. P. Sonnett[2] reported more specifically on "an apparent steady component of the interplanetary magnetic field". This component is also termed a "quiet time field" in ref. 1 which speaks of "The sudden increases in magnetic field strength during solar activity and the apparent existence of a quiet time field perpendicular to the ecliptic".

For convenience, we shall here denote the apparent quiet-time field vector by $\mathbf{B}_q$, the "sudden increases" or storm-time field vectors by $\mathbf{B}_s$ and the total field $\mathbf{B}_q + \mathbf{B}_s$ by $\mathbf{B}$. Also the field variation vectors observed on the Earth's surface during the main phase of a storm will be denoted by $\mathbf{F}_s$.

Although ref. 1 states that the quiet-time vector $\mathbf{B}_q$ is perpendicular to the ecliptic, neither reference states the sense of this vector. As a knowledge of this sense is essential to any theory of the origin of this quiet-time field, I shall present here arguments which appear to show that from the facts stated in refs. 1 and 2 and from the well-established facts concerning the direction and sense of terrestrial storm fields $\mathbf{F}_s$, we may infer that very probably the quiet-time field $\mathbf{B}_q$ is directed from the north to the south of the ecliptic.

These facts may be set out as follows in four groups, 1–4.

(1) The two graphs in Fig. 2 of ref. 1 which give as a function of time the average measured values of $\mathbf{B}_{\perp}$, the field component perpendicular to the ecliptic, and the magnetic *a*-indices recorded at Fort Belvoir (near Washington, D.C., and so in latitude 38° 53′ N.) during the solar event which commenced on March 31 and ended on April 3, 1960. These graphs are strikingly similar throughout and have their main peak values, 50γ and *a*-index 400, near the beginning of April 1.

(2) During this event *Pioneer* V was about 6 million km. distant from the Earth and both were about 150 million km. from the Sun, that is, *Pioneer* V was both well outside the influence of the Earth's magnetic field and near to the Earth within about 4 per cent of the Earth's distance from the Sun.

(3) The terrestrial storm field vectors $\mathbf{F}_s$, at medium and lower latitudes and during the main phase of a storm, are in general nearly parallel to the Earth's magnetic axis and directed from north to south, that is, $\mathbf{F}_s$ is nearly perpendicular to the ecliptic and directed from the north to the south. Also, in such latitudes the storm-time variations in the magnitude of the horizontal component of the Earth's field are in general much larger than those of the other two components (this could perhaps be verified by examination of the records at Fort Belvoir), and so the *a*-index is a statistically consistent measure of the magnitude F_s of the storm-field $\mathbf{F}_s$.

(4) In Fig. 2 of ref. 1 the value of the component $\mathbf{B}_{\perp}$ of the total field $\mathbf{B}$ was never less than the observed magnitude, $2 \cdot 5\gamma$, of the quiet-time field $\mathbf{B}_q$. Fig. 2 of ref. 2 shows that approximately the same fact was observed over the much longer period of 15 days, March 27–April 11, and during 22 other days.

On these four sets of facts we may base the following arguments:

(*a*) By (1), during the period of the solar event the Sun produced simultaneously a magnetic storm field $\mathbf{B}_s$ at *Pioneer* V and a magnetic storm field $\mathbf{F}_s$ at the Earth which vary together quite similarly in time. Since by (2) these two fields occurred in a region with radial dimensions only 4 per cent of its distance from the solar source of these fields it is very probable that they were parallel to one another. It is also probable that they both arose from an apparent parallel field $\mathbf{H}_s$ produced by the Sun throughout this region.

(*b*) By (3) and (*a*) it now follows that very probably $\mathbf{B}_s$ was nearly perpendicular to the ecliptic and directed from the north to the south.

(*c*) Since $\mathbf{B}_q$ was also perpendicular to the ecliptic it follows from (*b*) that $\mathbf{B}_q$ was very probably nearly parallel to $\mathbf{B}_s$. Hence the total field $\mathbf{B} = \mathbf{B}_q + \mathbf{B}_s$ was probably nearly perpendicular to the ecliptic, and so its magnitude would be given by the observed field $\mathbf{B}_{\perp}$ perpendicular to the axis of spin of *Pioneer* V, for this axis is nearly parallel to the ecliptic.

(*d*) If the sense of $\mathbf{B}_q$ had been opposite to that of $\mathbf{B}_s$ the magnitude of $\mathbf{B}$ and therefore also $\mathbf{B}_{\perp}$ would often have become much less than $2 \cdot 5\gamma$. Since this never occurred during the 37 days mentioned under (4), it follows that $\mathbf{B}_q$ always had the same sense as $\mathbf{B}_s$.

(*e*) It follows from (*d*) and (*b*) that very probably the sense of $\mathbf{B}_q$ was from north to south.

We can now summarize, as follows, the interplanetary findings of the magnetometer on *Pioneer* V: (1) The quiet-time field vector $\mathbf{B}_q$ was perpendicular to the ecliptic; (2) $\mathbf{B}_q$ was very probably directed from north to south; (3) the magnitude of $\mathbf{B}_q$ was about $2 \cdot 5\gamma$; (4) the storm fields $\mathbf{B}_s$ produced by the Sun were very probably perpendicular to the ecliptic and directed from north to south. The peak value of $\mathbf{B}_s$ was about 50γ.

In their discussion of the findings labelled (1) and (3) above, Coleman *et al.*[2] refer to the "serious discrepancies between the implications of these observations and present models of the interplanetary field". It is therefore interesting to note that the findings (1) and (2) agree exactly with the direction and sense of the magnetic field vector $\mathbf{H}_r$ set up near the Earth as a result of its orbital motion about a negatively charged Sun. It should also be noted that this theoretical field $\mathbf{H}_r$ was deduced from a general hypothesis about the charges on stars similar to the Sun, which was considered in three papers[3–5] submitted for publication before the launching of *Pioneer* V.

For a comparison of the finding (3) above with theoretical estimates of the magnitude of $\mathbf{H}_r$ reference may be made to the addendum in the third publication[5], where it is shown that the observed quiet-time field is about 55 times smaller than the nearest estimate made without considering the possible diamagnetic effects of any plasmoid in which *Pioneer* V could have been immersed[6].

It is also interesting to note that the largest observed storm field vector $\mathbf{B}_s$ appears to agree in direction, sense and order of magnitude with the theoretical vector $\mathbf{H}_r$, as calculated in the same addendum.

Despite this encouraging partial confirmation of the solar charge hypothesis it is evidently necessary to test it thoroughly by means of magnetometers and electrometers carried in space probes moving in a number of different orbits and using the appropriate Lorentz transformations as well as any theory of electromagneto-ionic media which can be developed for this purpose.

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GEOPHYSICS

Palæomagnetic Evidence Relevant to a Change in the Earth's Radius

Interest in the hypothesis that the Earth's radius has increased during geological history has been renewed in recent years because of several sets of independent observations and interpretations. From studies of the deformation of mountain ranges and the distribution of faults and oceans, Carey[1] proposes an increase in the Earth's area of 45 per cent since the Palæozoic era. Heezen[2] similarly interprets submarine topography as indicating that the oceans may be immense rift valleys formed by a pulling apart of the continents as the Earth expanded. Using a different approach, Egyed[3,4] infers a rate of increase of the Earth's radius of 0·4–0·8 mm. per year. This calculation is based on a decrease in the total amount of continental area covered by oceans during the past 400 million years, as determined palæographically. Egyed[4] has also pointed out the desirability of using palæomagnetic data to test this hypothesis.

During the Permian period, the Earth's magnetic field was remarkably steady. Palæomagnetic studies of western European rocks of this age have a high measure of internal consistency, and these western European data, together with the results from a recent palæomagnetic study by Gusev[5] of 1,600 igneous rock samples from Siberia, now make it possible to set some limits for changes in the Earth's radius during the past 235 million years.

In an expansion of the Earth as envisaged by Carey[1], Heezen[2] and Egyed[4], the continents do not increase in area; hence, the distance between any two points on a stable part of one continent remains constant, and as the Earth's radius increases the geocentric angle between the two points decreases. If the configuration of the ancient geomagnetic field is known, then this hypothesis can be tested with palæomagnetic results. In the present analysis a dipole configuration is used; this assumption is reasonable since palæomagnetic studies have firmly established that during the past million years, at least, the geomagnetic field was very closely dipolar when averaged over 10^4 years[6]. No assumption about coincidence of the ancient magnetic and rotation axes is required.

The method used for finding the ancient Earth radius from palæomagnetic data is essentially the one originally used by Eratosthenes in the third century B.C. to obtain the present Earth radius from astronomical data. If contemporaneous palæomagnetic data from two localities on the same stable continental block have different inclinations I_1 and I_2, so that the two localities are on different circles of geomagnetic latitude, and if the ancient geomagnetic field was dipolar, then the ancient Earth radius R may be found from:

$$R = d/[\cot^{-1}(\tfrac{1}{2}\tan I_1) - \cot^{-1}(\tfrac{1}{2}\tan I_2)]$$

where d is the distance measured along the Earth's surface between the two geomagnetic latitude circles[4].

In order to evaluate the Earth's radius during the Permian period, data were analysed from palæomagnetic studies of 16 igneous and sedimentary formations from western Europe and 5 igneous formations from Siberia. Representative sampling areas in western Europe and Siberia are shown in Fig. 1, together with the virtual geomagnetic poles (fictive geocentric dipoles that would give rise to the palæomagnetically measured field direction at each locality) corresponding to the 21 data used in the present analysis. Also shown are other poles based on western European data which could not be used in the present analysis because inclination values were not published. The data analysed include the study by Creer[7] of the Exeter traps, by DuBois[8] on the Mauchline sediments and lavas; the various studies of the Esterel volcanic rocks by Roche[9], Rutten *et al.*[10], and As and Zijderveld[11]; the study by Rutten *et al.*[10] of the trachyandesites of the Oslo graben; that of Armstrong[12] of the Ayrshire kylites; the studies by Nairn[13,14] of the Niedeck porphyry, Montcenis sediments, Saint-Wendel sediments, and Rotliegendes sediments and lavas; and the study by Gusev[5] on five igneous formations from Siberia. Although the geological age of the Siberian formations is subject to some uncertainty, Gusev[5] concludes that their radiometrically determined age of 250 million years is probably correct. This would correspond to mid-Permian on the new Kulp[15] geological time-scale, which incorporates recent Russian radiometric dating results.

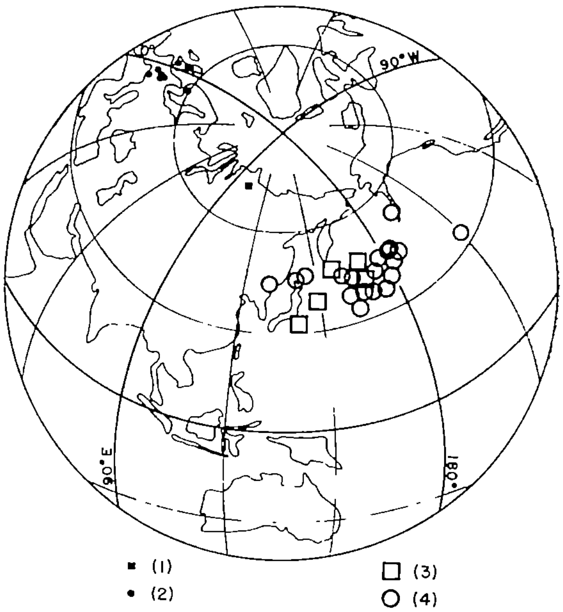

Fig. 1. Virtual geomagnetic poles calculated from western European (4) and Siberian (3) palæomagnetic data, and representative sampling localities in western Europe (2) and Siberia (1)

The method of calculation is by pairing each of the 16 inclinations from western Europe with each of the 5 inclinations from Siberia for a total of 80 determinations of R. To evaluate d for each pair of inclinations, small circles were constructed passing through each sampling locality about the average Permian virtual geomagnetic pole position as the common centre; d is the linear distance on the surface of the sphere between appropriate circles. The average Permian pole position was found by statistically analysing the 21 virtual geomagnetic poles, using Fisher's[16] method.

The average of the 80 values thus calculated for the Earth's radius during the Permian is:

$$R_{\text{Permian}} = 6{,}310 \text{ km.}$$

The standard deviation of individual determinations is 1,080 km. and the standard deviation of the mean (21 independent data) is 230 km. This result is subject to an additional uncertainty if, as is quite possible, the average magnetic pole position moved during the Permian and if the time-intervals spanned by the western European data and those from Siberia are not precisely the same.

Comparing these results with the present Earth radius of 6,370 km., we come to the following conclusions. The average Permian magnetic field, as seen from two sampling areas almost 5,000 km. apart on the Eurasian land-mass, shows no significant departure from the dipolar configuration and is perfectly consistent with a Permian Earth radius equal to the present one. An increase in the Earth's radius as slow as that suggested by Egyed (0·4–0·8 mm. per year or 94–188 km. in the past 235 million years) is neither confirmed nor rejected by the palæomagnetic data now available. Although precise statistical confidence limits cannot be assigned to the mean value of 6,310 km. for the Permian Earth radius, an increase since the Permian as large as the 1,100 km. required to produce a 45 per cent increase in area, as suggested by Carey, appears unlikely.

Publication of this communication is authorized by the Director of the U.S. Geological Survey.

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RADIOPHYSICS

Enhancement of Ionization in the *E*-Layer due to Solar Flares during the International Geophysical Year

INCREASES in the ionization of the *E*-layer have been reported after the occurrence of individual major solar flares[1,2], and an analysis by Bibl[3], for a three-month period in 1950, has shown that all increases in the critical frequency of the *E*-layer of more than about 0·2 Mc./s. above the monthly median value can be attributed to the effects of solar flares. Bibl considered only the deviations of the critical frequency from its monthly median value, and a similar analysis for 8 flares in the period 1951–56 has also been made by Taubenheim[4]. No detailed comparison has so far been made between the critical frequency after a flare and its value before the flare. Recently, in the course of other work, Minnis and Bazzard[5] have examined the hour-to-hour changes in the values of the *E*-layer critical frequency (*foE*) at Slough for the period of the International Geophysical Year. These data have been used to study the changes in *foE* associated with the occurrence of all the flares of importance 2+ and 3 which were listed in the CRPL *F*-Series Bulletins (Part *B*) and which occurred during the period 0800–1500h. U.T.

The value of *foE* for the observation at the hour immediately preceding the commencement of each flare and that for the two succeeding hours were examined in relation to the normal values for these hours. The normal value for each hour of any day was assumed to be that given by the best-fit line drawn through the points on a log *foE*–log cos χ plot for the period 0900–1500h. L.M.T. on the same day. The ratio of the observed to the normal value of *foE* was calculated for each of the three hours mentioned above. For each flare, the ratios for the two post-flare hours were then normalized by dividing by the corresponding pre-flare ratio.

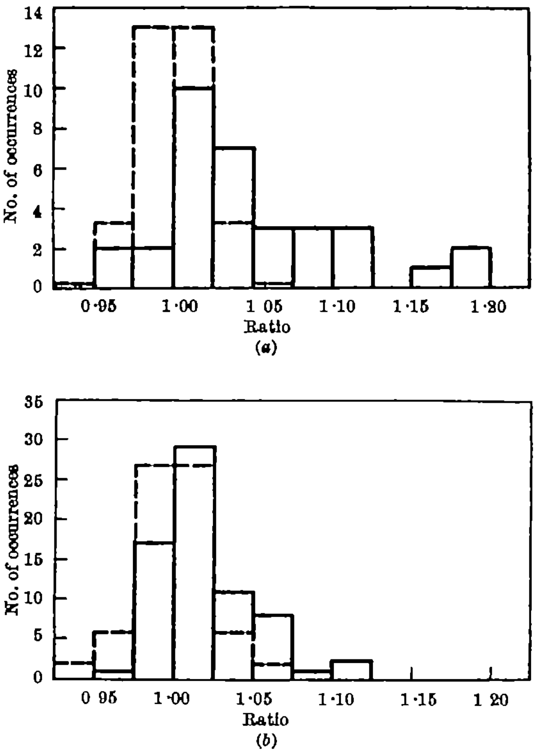

Fig. 1. Distribution of variations in *foE* about its normal value. Full line, up to 2 hr. after a solar flare; dotted line, no flare. (*a*) Class 3 flares; (*b*) Class 2 + flares

The distribution of the normalized post-flare ratios for Class 3 flares is shown in Fig. 1*a*. Twenty flares have been considered which gave 33 post-flare observations of *foE*. Some post-flare observations are not available, mainly owing to the high ionospheric absorption immediately following these flares. Minnis and Bazzard[5] have shown that the standard deviation of the hourly values of *foE* about the best-fit line was approximately 2 per cent at Slough during the International Geophysical Year. The expected distribution of ratios when there are no flares can thus be calculated and is also shown in Fig. 1*a*. Application of the χ^2-test to the distributions shows that the observed increase in the frequency of occurrence of high ratios during the two hours after a flare is significant. A similar result was obtained from an analysis of 41 Class 2+ flares which gave 69 post-flare observations of *foE*. The corresponding distributions are shown in Fig. 1*b*.

The distributions for the two classes of flare are also significantly different from one another. For Class 3 and Class 2+ flares, the mean values of the critical frequency ratios are 1·05 and 1·02 respectively, while the corresponding mean times of the observations are 65 min. and 59 min. after the commencement of the flare. It should be mentioned here that, owing to the extremely high absorption which often persists for a short time after the commencement of a flare, the number of missed observations of *foE* is greater during the earlier part than the later part of the post-flare period. For this reason, the mean ratios quoted above are probably less than they would have been if all the possible observations had been available, particularly for the more important flares.

An attempt was also made to estimate the rate at which the critical frequency returns to normal after a flare. It was not possible to measure this rate accurately as a function of time after the commencement of the flare. However, the mean rate of change of the critical frequency was found to be 3·4 per cent per hour for Class 3 flares and 1·4 per cent per hour for Class 2+ flares during the interval from approximately 25 to 120 min. after the start of the flare; this interval includes about 90 per cent of the observations of critical frequency. These observed rates of change of *foE* correspond to rates of change of electron density which are of the order of a thirtieth of those which would be expected if the layer were recovering normally from a sudden increase of ionization of the magnitude caused by the flares. It is assumed, therefore, that the observed rates of change of *foE* must represent the rate of change of intensity of the ionizing flare radiation incident on the *E*-layer. This conclusion is consistent with Taubenheim's results[4], which were, however, based on a smaller number of observations.

It is concluded from the foregoing analysis that, during the interval from about 25 min. to 120 min. after the commencement of a flare, there is a mean increase in the intensity of *E*-layer ionizing radiation which is at least 22 per cent for Class 3 flares and 8 per cent for Class 2+ flares. The mean rate of decrease of the intensity of ionizing radiation is approximately 15 per cent per hr. for Class 3 flares, and 6 per cent per hr. for Class 2+ flares.

The work described in this communication was carried out as part of the programme of the Radio Research Board and is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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Skip-Distance Ray-focusing in the Ionosphere

During the period March 1–April 6, 1960, the satellite *Sputnik* 3 passed over Britain at heights below 300 km., and on some sixty occasions the 20 Mc./s. signals were received at the Radio Research Station, Slough, when the satellite was close to or beyond the horizon, propagation being by 1/2-hop and 3/2-hop modes as has been reported for earlier satellites[1]. In the 1/2-hop mode the signal is reflected directly from the ionosphere to the receiving point, and, for the 3/2-hop mode, one ground reflexion precedes the ionospheric reflexion. Under these conditions, evidence has been obtained of strong focusing by the ionosphere of waves travelling along paths close to the skip-distance path.

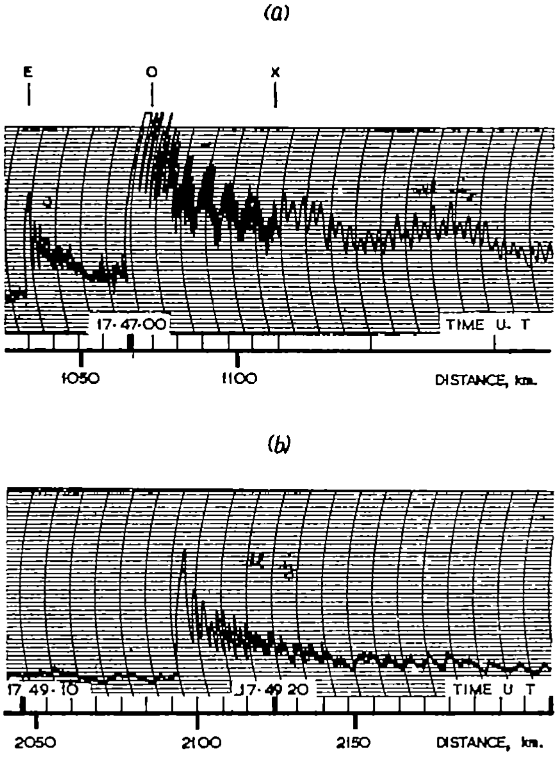

Fig. 1. Examples of skip-distance ray focusing 20 Mc./s. *Sputnik* 3 continuous wave transmissions, March 1, 1960. (*a*) 1/2 Hop; (*b*) 3/2 hop

For example, on near overhead transits, it was noticed that as the signal from the receding satellite began to fade out, a sudden burst of signal-strength occurred, which was often as strong as, and sometimes stronger than, the signal at nearest approach, and was followed by an oscillatory decay as illustrated in Fig. 1. The pitch of the audio-frequency note heard on the receiver due to the beating of the signal with a fixed reference frequency had a warbling characteristic during this period.

Fig. 1*a* shows an amplitude record of one such occurrence. Time marks at 1-sec. intervals are shown, together with the distance to the sub-satellite point. At point *X* the recording paper speed was increased five times. During this transit the satellite passed from north-west to south-east at 200 km. altitude, and the aerial used was a horizontal dipole oriented in a north-west–south-east line. The bursts of signal are attributed to the satellite crossing the 1/2-hop skip-distance boundaries: the first burst (*E*) occurring at the crossing of the boundary for the extraordinary wave, and the second, (*O*), 4 sec. later, at the crossing of the boundary for the ordinary wave. The higher-frequency oscillations (of the order of 10 c./s.) after the main burst are due to interference between the high-angle and low-angle rays, which may be received from a point beyond the skip distance; the depth of these oscillations decreases since the strength of the upper ray diminishes much more rapidly than that of the lower ray.

Appleton and Beynon[2] observed a similar phenomenon in 1946 when receiving fixed-frequency transmissions at Slough from a transmitter in Oslo. In this case events analogous to those described above were produced by the increase of skip distance due to a decrease of F-layer ionization. The theory of skip-distance ray focusing has been described by Bremmer[3], who gives a solution for the case of transmitter and receiver at ground-level, and shows a diagram somewhat similar to Fig. 1*a*. Using approximate ionospheric data obtained at the time of transit, a parabolic model, and the solution of Bremmer, a frequency of 10 c./s. has been calculated for the frequency of oscillation just after the main peak, increasing thereafter, for a transmitter moving away at ground-level with the satellite speed of 7·9 km./sec. The observed frequency is about 4 c./s., rising to 15 c./s. after 10 sec., which is in reasonable agreement with the calculated value. In addition, the record shows an oscillation of about 1 c./s., which is believed to be due to interference between the ordinary and extraordinary wave components.

Signals of the type shown in Fig. 1*a* have been obtained on the majority of close transits, except during the severe ionospheric storm of April 1–3, 1960. On many of the close transits, clear 3/2-hop skip-distance focusing has also been obtained. Fig. 1*b* shows the 3/2-hop peak which followed the 1/2-hop peaks shown in Fig. 1*a*; although two 3/2-hop peaks are usually present, only a single peak was observed on this occasion. In one case a clear 5/2-hop peak was also observed.

These observations provide confirmation of wave interference and focusing phenomena near the boundary of the skip zone, and are in general agreement with a related theoretical analysis of Bremmer.

This work forms part of the programme of the Radio Research Board and is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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PHYSICS

Standardization of Strong Electromagnetic Fields

THE possible harmful biological effects of high-intensity electromagnetic radiation have become of practical importance in recent years with the development of high-power radar and communication transmitters[1]. As a result, several instruments have been designed to measure total flux density in the frequency band 30 Mc./s.–30 Gc./s. (wave-lengths, 10 m.–1 cm.). In general, these devices indicate relative values only, and suitable techniques are therefore required by means of which they can be calibrated. Some comments on this problem are given in this communication.

In principle, standard flux densities of the specified value (1–20 mW./sq.cm.) can be established by radiating a known power from a transmitter under conditions which permit the calculation of the field strength or flux density at the receiver. This method is a convenient one, for example, at frequencies of the order of 10 Gc./s. with a horn-type aerial at the transmitter. If the horn has a gain of, say, 25 db. with respect to an isotropic radiator, a flux density of 10 mW./sq.cm. is produced at a distance of 3 m. (in a wave-front which is very nearly plane) using an oscillator of about 35 W. output. However, at frequencies in the range 100 Mc./s.–1 Gc./s. a similar procedure requires relatively large, directional aerials located well above ground on an open site, and transmitter powers of the order of a kilowatt or more. There is a need, therefore, for a standard laboratory technique operating at frequencies below about 3 Gc./s. and at power-levels of 10–50 W.

Some form of 'guided-wave' system presents an attractive solution to the problem. Apart from the advantages associated with the use of relatively low power-levels, such a technique would reduce possible radiation hazards to operating personnel to a minimum. Experiments have already been carried out at the Radio Research Station with these considerations in mind, and a summary of the basic principles and some preliminary results are given here.

A 'T.E.M.'-type wave is transmitted between parallel brass plates forming a large strip-line (3·5 m. long, 60 cm. wide and 10 cm. apart), terminated in an absorbing load. The input power (10–20 W.) is conveyed to the main structure by means of a 'coaxial to strip-line' transformer, followed by a taper section and a dielectric lens. The arrangement is somewhat similar to that adopted by El-Kharadly[2] for measurements on artificial dielectrics at a frequency of 3 Gc./s. A slotted section with a movable probe enables investigations to be made of the electric field distribution in both axial and transverse directions. An approximate value of the flux density in the parallel-plate section could be derived from a knowledge of the input power, but an indirect method is preferred for precision measurements. The latter technique makes use of a small probe and crystal detector calibrated by reference to a rectangular wave-guide transmitting a known power. If the total power flowing in a rectangular wave-guide propagating the dominant, H_{10}, mode is P watts, then the maximum electric field strength, E, at the centre of the broad face is given by:

$$E = (480\,P\,\pi\lambda_g/ab\lambda)^{1/2} \text{ volts/m.} \qquad (1)$$

where λ and λ_g are the free-space and guide wave-lengths respectively, and a and b are the internal dimensions of the guide in metres. If the voltage output of the probe is V for a field strength E in the rectangular wave-guide, then the same output, V, will be given by the same probe in the parallel-plate system when a flux density W is established, which is given by:

$$W = E^2/120\pi = 4P\lambda_g/ab\lambda \quad \text{W./sq.m.} \qquad (2)$$

The above procedure has been used in experiments carried out at a frequency of 1·2 Gc./s. The voltage standing-wave ratio ($E_{\text{max.}}/E_{\text{min.}}$) in the strip-line, when terminated by a hollow wedge-type load filled with powdered carbon, is 1·1 or less, and the transverse field distribution is approximately uniform. Devices calibrated in the strip-line have included crystal detectors and thermo-junctions surrounded by absorbing material. The results obtained to date suggest that an error of less than ± 1 db. can be expected at frequencies of the order of 1 Gc./s., and experiments are in progress to investigate the method at lower frequencies. In order to avoid the construction of a large rectangular wave-guide, a coaxial line terminated in a non-reflecting load could be used to calibrate the probe detector at frequencies of the order of one or two hundred megacycles per second.

It is important that the perturbation produced in the strip-line by the device being calibrated should be small. This should not often present any serious difficulty, however, since the monitoring devices being developed will normally have a small measuring probe remote from the main indicating equipment.

The work described above was carried out as part of the programme of the Radio Research Board. This communication is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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The 'Ballas' Form of Diamond

THE rare 'ballas' form of natural diamond is referred to occasionally in the literature. Ballas diamonds have been described by various authors as follows: (1) "Dense, globular aggregates of small, radially disposed diamond crystals"[1]; (2) ". . . a very hard, tough, globular mass of diamond crystals radiating from a common center"[2]; (3) ". . . a rare form of natural diamond with radial symmetry composed of apparently spherically grown layers . . ."[3]; (4) "Radial stalk-like formation of diamond crystals, or irregular growths of multiple crystals"[4].

These descriptions allude to the idea that a ballas has some type of radial structure in its crystal arrangement. Recently, I examined a polished section of a ballas with the view of determining its structure, since I could not find a photograph of such a section or imagine precisely what sort of structure would fit the published descriptions. The section was made about 0·5 mm. from the centre of a 0·70-carat stone. After a polishing operation, the stone was etched in molten potassium nitrate for 1 hr. at 700° C. A photomicrograph of the polished surface at 30 × is shown in Fig. 1. Residual scratches from the sectioning step are still visible. The centre of the sphere lies about 0·5 mm. below the × marked on the photograph

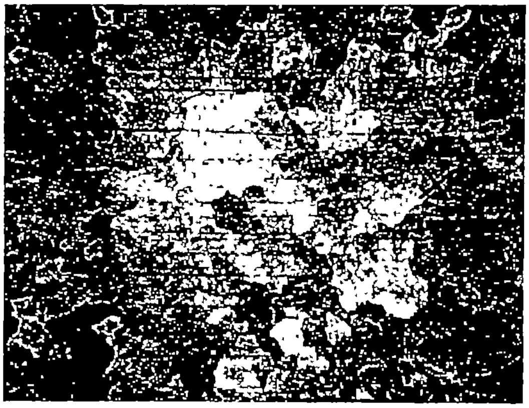

Fig. 1. Polished and etched section of a ballas diamond. (× 30)

Surprisingly, the many crystals making up the ballas do not appear to have any radial geometry. Such geometry should be revealed on a chordal plane so close to the centre of the sphere. This information indicates that previous descriptions are not precise or that the ballas at hand is an unusual variation. Part of description (4) above seems applicable to the findings. The irregularity of the crystal boundaries is an unusual feature of the structure. I should be glad to have any further information on this subject.

Williams, in his classic "Genesis of the Diamond", has shown sections of 'hailstone' bort which seems to fit description (3) above. It seems that 'hailstone' bort and ballas should not be confused as the same types.

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Frictional Oscillations

IT is generally considered[1,2] that vibrations excited by friction are due to a variation of friction with velocity, μ decreasing with increasing velocity, and in some cases (unpublished work) this is so. Vibrations in sliding systems can, however, be excited by other mechanisms. Consider a rigid strut $O'P$ held in an elastic pivot O' which in turn is attached to another strut $O'O''$, the pivot O'' being stiffer than O' (Fig. 1).

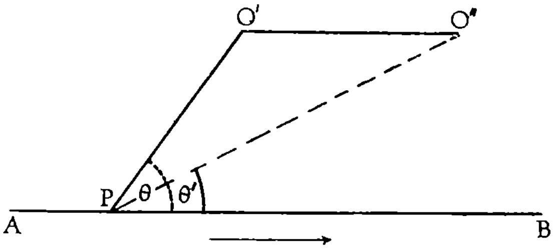

Fig. 1

The surface AB is moving past P in the direction shown and the coefficient of friction of the tip P is μ. The force F at P is given by $F = \mu L/(1 - \mu \tan\theta)$; so if $\mu = \cot\theta$, F is very large and 'spragging' occurs, that is, there is no slip between P and the moving surface AB. P is therefore displaced elastically in the direction of motion and θ increased slightly. As AB continues to move the elastic moment at the pivot O' opposing further deflexion of $O'P$ eventually becomes so large that $PO'O''$ becomes equivalent to a rigid strut $O''P$, O'' becomes the pivot point and θ is reduced to θ'. F falls accordingly so that the elastic energy stored in O' takes over and tends to swing P off the surface and slip occurs. The strut $O'P$ is therefore set vibrating and the surface AB is subjected to a periodically varying tangential force, and if AB is of suitable dimensions and elastic properties it can be set oscillating. Provided they are suitably mounted, solid bodies can be made to vibrate by a similar mechanism to the system of struts.

This sprag-slip motion can be demonstrated in several ways. If a strut OP pivoted in a rigid mount at O' is slid against a surface AB, $F=\mu L/(1-\mu\tan\theta)$ for values of $\cot\theta>\mu$; at $\cot\theta=\mu$ the strut breaks. If there are two pivot points $O'O''$, stick slip occurs at $\cot\theta=\mu$ and the oscillations of $O'P$ and $O'O''$ can be demonstrated with dial gauges or by moving smoked glass plates against probes attached to the struts. A very simple demonstration is to move a pencil held lightly at its extreme end and at its spragging angle across a sheet of paper.

The manner in which the exciter can set up vibrations in the opposing body can be demonstrated by holding a thin sheet of material edgewise against a rotating telephone bell. If the sheet is held at the spragging angle it is set in vibration and the bell emits a loud screeching sound. This sound contains a large number of harmonics, the vibrations are nonlinear and their frequency is determined by the natural frequency of the vibrator as well as that of the bell. In certain circumstances elastic deflexions in the bell itself reduce the friction force; the vibrations are then confined to the bell, which emits a loud relatively pure note. The frequency of this sound is almost the same as that of the sound emitted when the bell is struck.

I wish to thank the directors of Messrs. Ferodo, Ltd., for permission to publish this communication.

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Via Stockport.

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Excitation Functions of (d,α)- and (d,αn)-Reactions on Natural Tungsten

By irradiation in the Amsterdam cyclotron of natural tungsten with 22-MeV. deuterons, three species of radioactive tantalum were observed due to the following reactions: ^{184}W(d,α)^{182}Ta, ^{186}W(d,α)^{184}Ta and ^{186}W(d,αn)^{183}Ta.

The chemical separation of these nuclides was performed by selective liquid–liquid extraction with di-*iso*butylketone in hydrofluoric – hydrochloric acid medium in the presence of tantalum carrier[1].

Absolute tantalum activities were determined by means of a calibrated end-window Geiger tube. The results appeared to be in excellent agreement with those obtained with a 4π proportional counter.

The maximum deuteron energy of the cyclotron was computed by fitting an experimental relative excitation function of the ^{27}Al(d,αp)^{24}Na reaction with those obtained by Batzel *et al.*[2] and Moeken[3]. The maximum energy appeared to be 22 MeV.

The beam intensity was computed from the same reaction at the maximum deuteron energy, as absolute cross-sections for this reaction are also accurately known.

The cross-sections for the three reactions under consideration as a function of deuteron energy were computed from the measured tantalum activities, after adequate corrections for chemical yield, decay and counting efficiency of stacked tungsten foils irradiated in the internal beam of the Amsterdam synchro-cyclotron. The deuteron energy in each foil was calculated from the range–energy curve of tungsten computed from the Bethe–Livingstone equation[4]. The results are summarized in Table 1.

Table 1. CROSS-SECTIONS IN MILLIBARNS AS A FUNCTION OF DEUTERON ENERGY

Deuteron energy	^{186}W(d,α)^{184}Ta	^{184}W(d,α)^{182}Ta	^{186}W(d,αn)^{183}Ta
21	3 05	1 25	1·15
20	2·90	1·22	0·97
19	2 65	1·08	0·67
18	2·30	0·85	0·52
17	2·10	0·70	0·35
16	1·70	0·57	0·22
15	1·30	0·46	0·13
14	1·08	0·32	0 075
13	0 90	0·21	—
12	0·62	0 13	—
11	0·29	—	—
10	0 16	—	—
9	0·05	—	—

Acknowledgments are to due Prof. A. H. W. Aten, jun., I.K.O., Amsterdam, for valuable discussions during the performance of this work.

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GEOLOGY

Geology of the Floor of the Bristol Channel

A geological survey of the floor of the Bristol Channel was made from R.R.S. *Discovery II* between April 22 and May 2, 1960, using asdic[1–3] and bottom sampling equipment. Preliminary results are summarized in the accompanying map (Fig. 1). The northern part of the Channel is floored with superficial deposits except around Caldy Island. In the southern part there are extensive areas of exposed rock.

Upper Palæozoic rocks outcrop continuously from Hartland Point to Lundy Island. They include slates and quartzites, and the asdic records show a strong lineation orientated a few degrees north of west, which in some places appears to be bedding. The granite of Lundy Island does not extend more than a mile to the north, or half a mile to the south of the Island. We could not approach close to the east or west coasts on account of the sand banks, but believe that the outline of the Island corresponds closely to the boundary of the granite: no dykes were recognized near the Island.

Many west-north-west to north-west lineations were recorded by the asdic from the Upper Palæozoic rocks west of Ilfracombe, while eastward for 3 miles north-easterly lineations were found. At the Horse-shoe Rocks, a shoal surrounded by Upper Palæozoics about 4 miles north-west of Ilfracombe, we dredged dark green basic igneous rock comparable to a metamorphosed spilite. Skin divers have not yet established whether the spilite outcrops here, or whether it is derived from a superficial deposit.

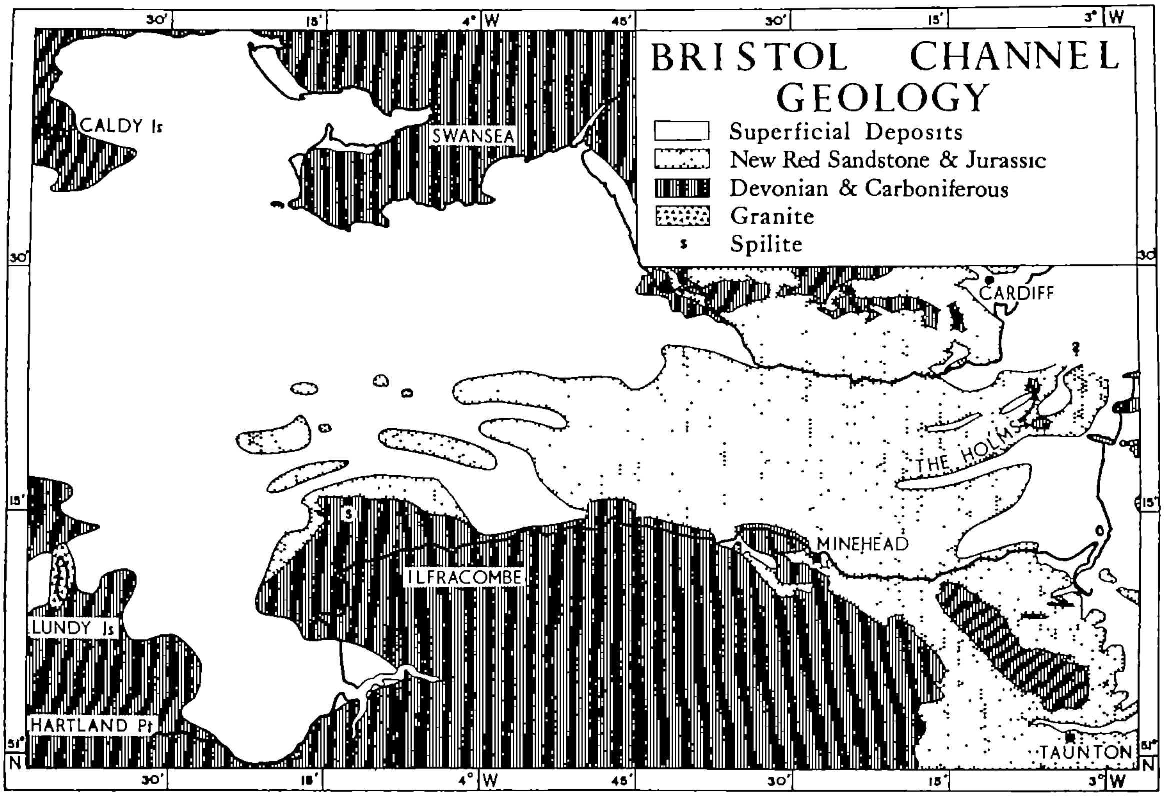
BRISTOL CHANNEL GEOLOGY
☐ Superficial Deposits
▨ New Red Sandstone & Jurassic
▥ Devonian & Carboniferous
▨ Granite
s Spilite
CALDY Is
SWANSEA
CARDIFF
THE HOLMS
MINEHEAD
ILFRACOMBE
LUNDY Is
HARTLAND Pt
TAUNTON
30'
15'
4° W
45'
3° W
51° N

Fig. 1

From the Holms westward to beyond Ilfracombe the floor of most of the channel is composed of Liassic rocks lying nearly horizontal: these approach very close to the southern shore west of Minehead. In some places there is New Red Sandstone between the Upper Palæozoics and the Lias, but a continuous strip of New Red Sandstone is not proved.

Two asdic reconnaissance lines run between Steep Holm and Flat Holm suggest that the intervening channel is floored by Mesozoic rocks, so that these Carboniferous masses were already isolated in Mesozoic times, as supposed by Strahan and Cantrill[4].

Between Lundy Island and Pembrokeshire the superficial deposits are continuous, except for Palæozoic outcrops near the Welsh coast. Westward extension of the Mesozoics to connect with the outcrops off north Cornwall reported by Day[5] is therefore unproved, but if such a connexion exists it must lie to the north of Lundy.

Detailed analysis of the findings is in progress and will be published elsewhere at a later date.

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METALLURGY

An Unusual Feature of the Fusion Welding of Niobium to Vanadium

Theilacker, Baugh, Kasburg and Stermon[1] observed apex-shaped boundaries between the fusion zone and parent plate in zirconium–hafnium welding, but offered no satisfactory explanation.

During some recent investigations into the fusion welding of niobium to vanadium, a similar but more pronounced effect was noticed at the boundary between niobium and the fusion zone. This 'tongue effect' is illustrated in Fig. 1.

A micro-hardness traverse carried out through the tip of the tongue showed that there was no gradation in hardness as the boundary was approached on either side. The change in hardness was sudden and a

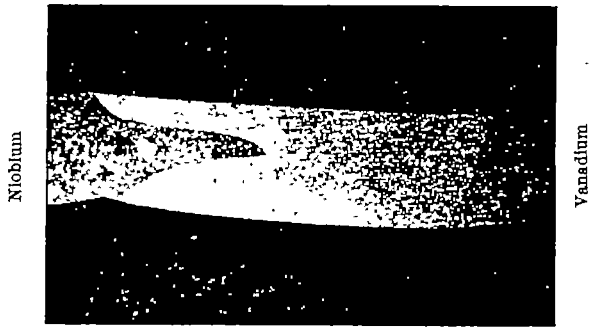
Niobium
Vanadium

Fig 1. Plate 1/16 in. thick

constant level persisted on both sides of the interface. The niobium parent plate had a hardness of 140 V.P.N. and the fusion zone had a hardness of 350 V.P.N. This indicated that there was virtually no diffusion layer showing a gradual fall of vanadium content in the solid niobium, but an extremely sharp division between the niobium and the fusion zone material. The same hardness conditions applied to the vanadium side of the weld.

Micro-hardness tests carried out across the tip of the tongue and parallel to the interfaces gave results in keeping with the above conditions.

The sectioning of further specimens has shown that at electrode traverse-rates of 4–5 in. per min., and given currents, or similarly at currents as low as 40 amp. and given traverse-rates, the tongue was much more blunt and under-developed. As the current increased or the traverse-rate decreased there was a definite trend from the blunt form to the sharp form of Fig. 1. In addition, it was noticed that at the start of a weld, it was necessary to melt vanadium first before the niobium became molten, at the currents used, and in fact a true bond would not form until the vanadium had run into the niobium and formed an alloy. Once this had happened welding appeared to continue normally. Thus it would seem that the temperature of the fused metal was below the melting point of niobium (2,420° C.) but above that of vanadium (1,860° C.). It was found also that in order to weld niobium to niobium, 30 per cent higher currents were required than to weld vanadium to vanadium for a similar bead width. This would be expected, when considering melting points and conductivities of the two metals.

Thus the evidence suggests that the blunt tongue is an early stage in the development of the sharper outline. This sharp contour was reached fairly quickly in the series and then was a constant feature. For example, a current of 70 amp. was used in the series of welds where traverse-rates were varied from 4 in. per min. to ½ in. per min. At a rate of 4 in. per min. the tongue was only partially formed, but at 2½ in. per min. it was complete.

It is considered that the mechanism of formation of the tongue is one of dissolution of niobium in weld metal.

A more complete treatment of this aspect will be published elsewhere.

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Effect of Air Pressure on the Fatigue of Lead and Aluminium

It is known that the fatigue strengths of lead and aluminium are affected by the presence of the atmosphere during fatigue stressing[1–4]. The present communication reports the results of experiments on the dependence of the fatigue life of lead and aluminium on air pressure at room temperature, 22° C.

Specimens were prepared from high-purity rolled strip (99·999 per cent lead, and 'super pure' aluminium), and, after annealing, were fatigued in reverse plane bending at the rate of 500 cycles/min.

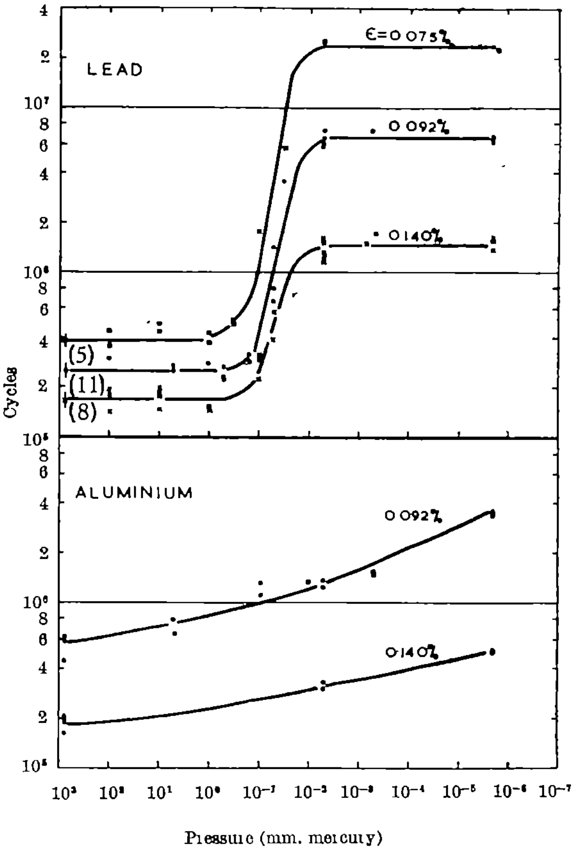

Fig. 1. Variation of log fatigue life with log air pressure. In the top figure (for lead) the figures in brackets refer to the number of specimens tested at normal atmospheric pressure; all other points are plotted

The influence of air pressure on the fatigue life at various constant strain amplitudes is shown in Fig. 1. The curves for aluminium indicate a steady increase in life with decrease of air pressure which is in agreement with the dependence on air pressure of the fatigue life of aluminium and copper found recently by Wadsworth and Hutchings[3].

In contrast, each of the three curves shown for lead displays three distinct ranges: (*a*) a high-pressure range in which the life is constant (atmospheric pressure to approx. 0·15 mm. mercury); (*b*) an intermediate range in which the life increases rapidly with decreasing pressure (approx. 0·15–5 × 10^{-3} mm. mercury); and (*c*) a low-pressure range in which the life is unaffected by further reduction of pressure (approx. 5 × 10^{-3}–2 × 10^{-6} mm. mercury). The stepped form of the curves for lead is thought to be associated with specific changes in the nature of the fatigue deformation produced by variations in the rate of reaction between the gas and the metal at different gas pressures. For example, in the high-pressure range (*a*) specimens failed by intercrystalline cracking whereas in the low-pressure range (*c*) the intercrystalline cracking was suppressed and failure occurred by extensive slip deformation[4]. On the other hand, in the case of aluminium[3], it appears that the presence of the air only affects the rate of propagation of cracks and does not affect the type of cracking. However, it may well be that the fatigue behaviour of aluminium at elevated temperatures would show a closer resemblance to that of lead at room temperature and, conversely, that the fatigue behaviour of lead at liquid-air temperature would show a closer resemblance to that of aluminium at room temperature.

This work formed part of the research programme of Broken Hill Associated Smelters Pty. Ltd. and was carried out under the supervision of Prof. J. Neill Greenwood, to whom thanks are due for helpful discussions.

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RADIATION CHEMISTRY

Labelling of Organic Compounds by Mercury-photosensitized Reaction with Tritium Gas

The widely employed Wilzbach's tritiation method is based on the exchange reaction between an organic substance and tritium gas induced by radiation from several curies of T_2 (ref. 1).

In order to increase the exchange-rate and to reduce the amount of tritium required, several techniques have been developed which employ an external energy source for promoting the labelling process: electrical discharges[2-5], γ-rays[6], microwaves[5] have been used. Recently[7] the tritiation of two polymers by ultra-violet irradiation in the presence of tritium has been described.

We report here a simple labelling technique, based on the mercury-photosensitized exchange between tritium gas and an organic compound, which, in principle, allows the tritiation of all the substances, including those which do not absorb in the ultra-violet region. In using this method, a silica ampoule containing the organic sample and tritium gas, with a small amount of mercury, is irradiated at room temperature with a low-pressure mercury lamp. The energy of the 2537 Å. resonance line is absorbed by the mercury vapour which, as pointed out by Kaplan[8] in a detailed study on the reaction of unsaturated hydrocarbons with tritium, causes the excitation and the dissociation of both tritium and organic molecules.

Table 1

Compound	Run No.	T_2 activity (mc.)	Irradiation time* (hr.)	Specific activity in pure compound (mc./gm.)	Total activity in pure compound (mc.)
Toluene	1	10±2·5	120	1·78±0·01	1·58
Toluene	2	10±2·5	96	0·730±0·006	1·26
Toluene, blank†	—	10±2·5	—	0·0053±0·0002	—
*Cyclo*hexane	3	10±2·5	120	0·223±0·002	0·356
*Cyclo*hexane, blank†	—	10±2·5	—	0·0005	—

* The irradiations were performed at 25° C., tritium gas employed having been diluted with pure hydrogen.

† In the blank runs the tritium gas and the hydrocarbons were in contact for a period of 120 hr. without ultra-violet irradiation.

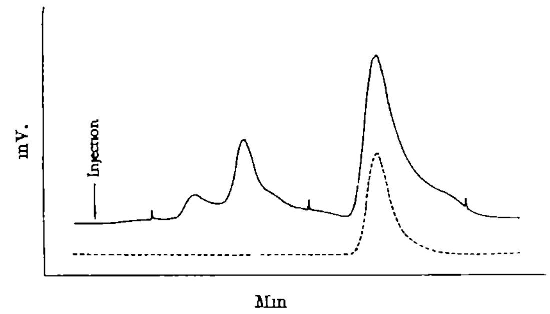

Fig. 1. Gas chromatographic (- - -) and radiochemical (——) analysis of crude, tritiated toluene. Carrier-free by-products, possibly hydrogenated toluenes, are detected from their radioactivity

The interaction of the reactive entities produced in this primary process ensures, among other reactions, an efficient tritium exchange including those cases where the organic compound does not exhibit absorption bands in the ultra-violet region. The application of the above technique to the preparation at tritiated toluene and *cyclo*hexane is briefly described. Table 1 summarizes the experimental conditions employed and the activity found in the pure, tritiated hydrocarbons.

A low-pressure model *NK* 20/40 Hanau resonance lamp was used for all the irradiations. A simultaneous gas-chromatographic and radiometric analysis of the crude toluene and *cyclo*hexane demonstrated the presence of several carrier-free radioactive by-products (Fig. 1).

An efficient radiochemical purification of the tritiated substances was achieved by preparative-scale gas chromatography. Columns 3 m. long, packed with di-*n*-decyl-phthalate on 'Celite *C*22' were employed, using nitrogen as carrier gas. The column temperature was 85° C. for the toluene and 70° C. for *cyclo*hexane.

The gas-chromatographic steps were repeated until a simultaneous mass and activity analysis failed to detect radioactive contaminants in the purified samples, the activities of which were measured in 250 ml. ionization chambers, connected to a vibrating-reed electrometer, as described by Wilzbach, Kaplan and Brown[9].

The results in Table 1 demonstrate that comparatively high percentages of the T_2 activity have been incorporated into the labelled compounds, including *cyclo*hexane, which does not absorb in the ultra-violet region.

To evaluate the efficiency of the present method, it may be pointed out that, with Wilzbach's technique, an activity of at least 500 mc. of T_2 must be employed to introduce the same tritium activity into pure toluene, in an equal exchange time. In this way, the higher efficiency of the mercury-photosensitized exchange method allows the preparation of labelled samples with a considerable saving of tritium activity and/or exchange period.

From the preliminary results obtained, it seems that the simple method described could be usefully employed as a general technique for the labelling of organic compounds.

Two mechanisms are conceivable to explain the mercury photosensitized exchange:

(1) Excitation and dissociation of gaseous tritium by $^3P_1$ mercury atoms, the energy of which is sufficient to dissociate the T_2 molecules; the tritium atoms can afterwards react with the organic substrate; (2) excitation and dissociation of organic molecules which can interact with tritium atoms or molecules.

More detailed information will be given later.

We are indebted to Prof. G. Giacomello for the helpful discussions.

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A 'Dry' Mounting Autoradiographic Technique for the Localization of Water-soluble Compounds

ONE of the disadvantages of the stripping film autoradiographic technique is that the film is floated on a water surface and apposed to the radioactive material by dipping the slide into the water and picking up the film so that its under surface (the emulsion layer) is in direct contact with the specimen. During this mounting procedure (called here the wet technique) and subsequent drying period, which may be several hours, there is opportunity for water-soluble compounds to diffuse out of the specimen, or to leave their original site and be absorbed elsewhere[1,2].

In an attempt to define the metabolic pathway of labelled precursors of deoxyribonucleic acid using either phosphorus-32 or tritium compounds, it became apparent that the soluble intermediates were, in fact, readily lost during the conventional autoradiographic procedures. The use of vacuum drying, freeze substitution or other methods of cell fixation did not prevent a considerable loss of phosphorus-32 from nuclei afterwards placed in aqueous solutions; autoradiographs of nuclei using these methods of fixation and the wet autoradiographic technique revealed some isotope definitely associated with nuclei, but much more was randomly distributed throughout the extranuclear portion of the smear. In some cases, there was relatively little radioactivity evident in the emulsion above the smear, but more encircling it, suggesting by the pattern that diffusion of isotope from the smear had occurred.

A direct mounting of dry, rectangular (1·5 × 3·5 cm.) strips of autoradiographic stripping plate (*AR.* 10) (Kodak, Ltd.) film emulsion to smears of nuclei from rat thymus glands on histological glass slides was achieved by the simple process of chilling the specimen in a refrigerator at 0–5° for 2–3 min., removing the specimens to the high humidity of the dark room, and immediately fixing the stripped emulsion to the specimen slide by firm even pressure with the thumb. It is assumed that the precipitation of a slight amount of moisture on the cold slide together with the pressure and warmth of the thumb permitted the firm bonding of the stripping film emulsion to the specimen and the slide which had been previously coated with gelatin ('subbed'[1,2]). Even without chilling the slide it was possible to mount dry emulsion to slides of nuclei by heavy thumb pressure although more artefacts were noted with this method.

To avoid the possibility of radioactive contamination of the specimen by the thumb, pressure can be applied to the dry film by means of a large cover-slip or another glass slide.

The direct 'dry' mounting gave rise to firm bonding of the film and specimen so that the film withstood photographic processing and staining procedures and remained tightly bound.

Rat thymus nuclei were prepared in ionic and sucrose media[3]. They were fixed either by rapid lyophilization in a desiccator or by freezing in *iso*-pentane at liquid-nitrogen temperature and dehydrating in ethanol at solid carbon dioxide temperature for 3–7 days[4]. The latter were mounted by allowing the slide to warm at room temperature for a minute or two until most of the alcohol had evaporated and then fixing the dry film emulsion directly to the smear with thumb or cover-glass pressure. The lyophilized smears were chilled and the emulsion similarly applied.

By this direct dry mounting technique we have been able to show the presence of phosphorus-32 mainly in the nuclei and less in the extranuclear area of the smear whereas the standard wet mounting procedure revealed a diffuse distribution of radioactivity throughout the smear regardless of whether formalin or Bouin's fixative, vacuum drying, freeze-substitution, oven (90°) or room temperature fixation was used. Autoradiographs of nuclei from rat thymus gland after intramuscular injection of ^{3}H-deoxycytidylic acid showed more emulsion grains over the nuclei at 10 and 30 min. after injection than did corresponding nuclei prepared by the standard wet mounting process. Redispersion of nuclei in ethanol after isolation in sucrose or ionic media and 'dry' mounting also resulted in a higher nuclear than extranuclear concentration of phosphorus-32 when compared to the oven or room temperature drying or vacuum dehydration followed by wet mounting. The resolution attained by the use of tritium was not impaired[5].

Attempts are now being made to apply the 'dry' mounting technique to tissues fixed and cut in the cryostat[6,7] and to those cut in the cryostat and dehydrated by freeze-substitution methods[4].

Since satisfactory autoradiographs have been obtained by this dry mounting technique, it is likely that the method may also prove useful in similar work with other cell components.

A more complete account of the results given by this method will be described elsewhere. Our thanks are due to the Rockefeller Foundation for financial

assistance and to the Commonwealth Fund for a research fellowship to one of us (P. J. F.).

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CHEMISTRY

Rates of the Reactions of Nitrogen Atoms with Oxygen and with Nitric Oxide

The reactions of nitrogen atoms with oxygen and with nitric oxide are of considerable interest, since whereas both processes (1) and (2) are exothermic:

$$N + O_2 = NO + O \quad \Delta H^0 = -32{\cdot}8 \text{ kcal./mole} \quad (1)$$

$$N + NO = N_2 + O \quad \Delta H^0 = -75{\cdot}1 \text{ kcal./mole} \quad (2)$$

the former reaction is very slow at room temperature, while the latter is extremely rapid. Kistiakowsky and Volpi[1] obtained a value of 2×10^{13} exp(-6200/R.T.) cm.^{3} mole^{-1}sec.$^{-1}$ for k_1 from a mass spectrometric investigation of the reaction between nitrogen atoms and oxygen molecules in a stirred reactor between 390° and 520° K. while a value of $1{\cdot}7 \times 10^{13}$ exp($-7{,}500$/R.T.) can be obtained from Kaufman's[2] work on the oxygen-sensitized decomposition of nitric oxide between 1,500° and 1,700° K. No direct determination of k_2 appears to have been reported, although values of 2×10^{11} (ref. 3) and a lower limit of 5×10^{13} cm.^{3} mole^{-1}sec.$^{-1}$ (ref. 1) have been recorded.

In the present work, we have studied the reaction between nitrogen atoms and oxygen molecules in the temperature range 412–755° K. Active nitrogen containing about $\frac{1}{4}$–$\frac{1}{2}$ per cent nitrogen atoms was produced by passing highly purified nitrogen through a 20 Mc./sec. electrodeless discharge. Active nitrogen flowed from the discharge through a 19 mm. internal diameter quartz reaction tube enclosed in an electric furnace. This tube had four sealed-in mixing jets spaced at 30-cm. intervals. Oxygen at known flow-rates was introduced at the first jet, and the decay of the nitrogen atom concentration along the tube was determined by titration of the nitrogen atoms at the three downstream jets, and at the first jet (before addition of oxygen). In the titration method[1,4–6] the end-point is observed as a sharply defined extinction point between the blue nitric oxide β-emission and the green air afterglow continuum with increasing nitric oxide flow-rate. This titration 'end-point' was taken to occur at the same nitric oxide flow-rate as the minimum current of a photomultiplier which observed the light emitted from the flowing gas downstream from the fourth jet. The nitrogen atom flow-rate was equal to that of nitric oxide at the end-point.

It was found that the rate of removal of nitrogen atoms was first order with respect to both nitrogen atom and oxygen molecule concentration and that two nitrogen atoms were removed for each oxygen molecule reacted, since reaction (2) is much more rapid than reaction (1). In this way a velocity constant of $8{\cdot}3 \pm 1{\cdot}0 \times 10^{12}$ exp($-7{,}100 \pm 400$/R.T.) cm.^{3} mole^{-1}sec.$^{-1}$ was determined for k_1 over the temperature-range 412–755° K. The rate constants calculated from this expression agree with the rates observed by Kistiakowsky and Volpi[1] and by Kaufman[2] to within the combined experimental errors of the observations.

At the higher temperatures used in our experiments and at high oxygen flow-rates (5–50 times the nitrogen atom flow-rate) a weak air afterglow spectrum was observed, and since this is known[7] to be due to the radiative process $NO + O = NO_2 + h\nu$, nitric oxide as well as oxygen atoms must be present in the reaction products. Under these conditions the rates of reactions (1) and (2) are more nearly comparable than at the lower temperatures and oxygen flow-rates used in the study of the kinetics of the overall reaction, and an appreciable amount of nitric oxide remains when all the nitrogen atoms have been consumed. The concentration of nitric oxide in the effluent gas was always less than 10 per cent of the initial nitrogen atom concentration, and it can be shown that in these circumstances the concentration of nitric oxide produced (y_0) is given by $y_0/b = k_1/k_2$, where b is the concentration of oxygen. The small amount of nitric oxide formed does not decay appreciably along the reaction vessel since the slow reaction (3):

$$NO + O + M = NO_2 + M \qquad (3)$$

is followed by the rapid reaction (4) which regenerates NO from NO_2:

$$O + NO_2 = NO + O_2 \qquad (4)$$

The amount of nitric oxide in the effluent gas (typically 0·05 per cent of the total flow) cannot be determined by conventional analytical techniques, and the following method was adopted. It is known that the intensity of the air afterglow is directly proportional to the concentrations of nitric oxide and oxygen atoms, and is independent of inert gases, and in runs where the oxygen flow-rate is varied the relative nitric oxide concentrations are given by the corresponding light intensities observed by the photomultiplier since the flow-rate of oxygen atoms produced is independent of the flow-rate of molecular oxygen used (always a large excess). The photomultiplier was calibrated to read directly in nitric oxide flow-rate by observing the enhancement of the light intensity when nitric oxide (diluted with nitrogen) was added to the reaction system at known flow-rates between the point of oxygen addition and the photomultiplier. For constant oxygen flow-rate, the photomultiplier current plotted against the flow-rate of added nitric oxide–nitrogen mixture gave a straight line, the slope of which was independent of oxygen flow-rate; and hence the small decay of oxygen atoms between the mixing point and the photomultiplier was roughly the same for all oxygen flow-rates used here. Making a small correction for the photomultiplier dark current, values of the effluent nitric oxide concentration (y_0) were thus obtained in runs where the flow-rate of oxygen (b) was varied. Plots of y_0 against b gave satisfactory straight lines with slopes equal to k_1/k_2. Nitric oxide flow-rates down to 3 mμmoles/sec. were measurable with good accuracy by the technique described.

k_2 was determined as $3\cdot0 \pm 0\cdot6 \times 10^{13}$ exp$(-200 \pm 700/\text{R.T.})$ cm.^{3} mole^{-1}sec.$^{-1}$ over the temperature-range 476–755° K. Thus, a direct determination of a velocity constant equal to about one-fifth of the bimolecular collision-rate has been made. The difference between the two frequency factors of reactions (1) and (2) appears to be greater than the combined errors of the determinations. This difference is of interest, since it cannot be explained on statistical grounds.

A full account of this work will shortly be submitted for publication. We thank the Department of Scientific and Industrial Research for a grant to one of us (M. A. A. C.).

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Use of the Mercury Drop Electrode Method for Direct Evaluation of Minute Amounts of Lead in Urine

For the diagnosis of the degree of lead poisoning the determination of lead in urine is very important although not easy. In the case of chronic poisoning the amount of lead in urine is not much larger than in normal urine samples, in which the lead concentration is very low (up to 80 γ/l.). Therefore the direct determination of lead in freshly voided urine without decomposition of the sample by ashing or by digestion with oxidizing acid is difficult. Some attempts have been made to overcome these difficulties and to determine lead without preliminary concentration, but they have not given satisfactory results.

Chmielowski and Myślak[1], who applied Teisinger's method for the determination of lead in blood[2] to urine samples, could indeed determine lead directly, but because of the insufficient sensitivity of the polarographic method, only in quantities larger than 500 γ/l. This renders it possible to estimate lead in cases of acute poisoning solely or when a patient is treated by means of calcium versenate. Similarly, direct electrodeposition of lead on to a platinum electrode from unprepared urine carried out by Cooksey and Walton[3] has not been widely accepted.

The application of the hanging mercury electrode method[4] to such a problem offers some advantages. High sensitivity and the possibility of concentrating selectively lead, while the concentration of other components remains unchanged, enabled us to develop a method of direct determination of very minute quantities of lead in urine.

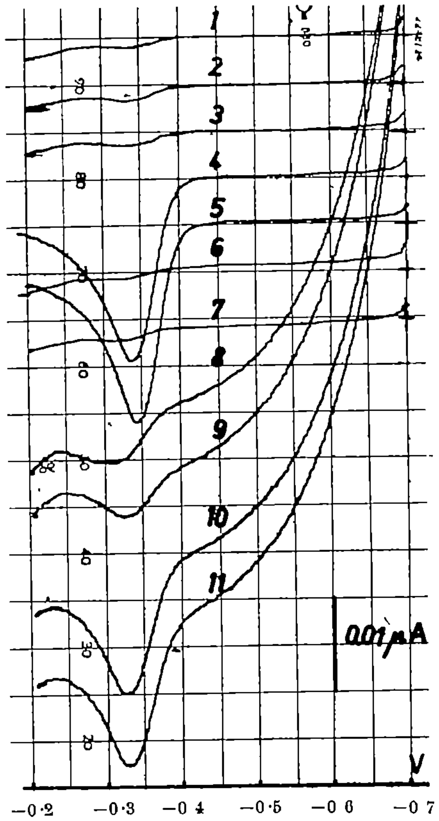

Fig. 1

The procedure is as follows: to the electrolytic cell which contains the hanging mercury drop electrode, salt bridge and small magnetic stirring bar, 10 c.c. of three times distilled water and 0·1 c.c. of specially prepared very pure concentrated hydrochloric acid were added. Oxygen was removed from the solution with hydrogen and then the preliminary electrolysis was carried out in an intensively stirred solution. During the electrolysis the potential of the cathode was kept constant at − 0·7 V. Stirring was stopped after 3 min. of concentrating electrolysis and the oxidation curves were recorded by means of a conventional polarograph polarized towards positive potential (− 0·7 to − 0·2 V.). In this way curves 1, 2 and 3 (Fig. 1) were recorded which show the degree of purity of the cell and added acid. A small minimum which appears at about − 0·35 V. corresponds to the oxidation of some traces of lead present in the acid and the electrolytic vessel. These traces can be determined by adding Pb^{2+} ions in such amounts that their concentration was 1×10^{-7} *M* (curves 4 and 5). Comparison of the curves 1, 2, 3 and 4, 5 shows that the concentration of Pb^{2+} ions in blank test solution was 5×10^{-9} *M*.

Again the electrolysis of a new sample of acidified water was carried out to show that lead previously brought in was sufficiently removed from the cell (curves 6 and 7). After removing this test solution, 10 c.c. of the urine was placed in the cell and 0·1 c.c. of acid was added, and then deposition as well as recording were repeated (curves 8 and 9). Due to the reduction of various organic components of urine, the curves recorded in acidified urine differ from those obtained in the test solution of acid. However, at the oxidation potential of lead the curve is sufficiently flat to observe distinctly the oxidation minimum of lead (curves 8 and 9). Curves 10 and 11 were recorded after adding a known quantity of lead forming a

1×10^{-7} M solution By comparing curves 8 and 10 it can be seen that the concentration of lead in urine was 5×10^{-8} M, that is, about 10 γ/l. The results obtained from twelve different 'normal' urine samples gave 10 3 γ/l. as a mean.

The oxidation minimum for lead corresponding to the same amount of the standard was much smaller in urine (curve 10) than in the acid (curve 4). This difference is caused by the greater viscosity of urine in comparison with the viscosity of the solution with acid added. The calibration curves describing the dependence of maximal intensity of oxidation curves on concentration of lead obtained for both solutions were linear, and this allows the use of the standard addition method.

These results show that lead can be directly and quantitatively determined not only in urine of persons poisoned with lead, but also in the 'normal' ones without any preliminary concentration and mineralization as a separate operation.

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Pyrazine-Metal Halide Complexes : New Types of Metal-Ligand Polymers

THE compounds[1,2] of pyridine with metal salts have been well investigated, but little has been reported concerning the corresponding pyrazine (Fig. 1) compounds. Pyrazines have a second (potential) donor nitrogen atom in the 4 position and are much weaker[3] bases than the pyridines. (Pyrazine $pK_a = 1{\cdot}1$; *cf.* pyridine $pK_a = 5{\cdot}2$.) It appears to attach a proton, or to quaternize at one nitrogen atom only, the second being deactivated[4]. Condensed pyrazines, for example phenazine[5], have been studied, but only two simple pyrazine complexes, namely, tetracarbonyl-*bis*(methylpyrazine)-chromium(O) and the corresponding molybdenum(O) compound, appear to have been reported[6].

Our investigation springs from an interest in the relative donor capacities of the two nitrogen atoms towards metals, in the effect of substituents on donor capacity, and in the potential value of these complexes for the preparation of semi-inorganic (metal–ligand–metal) polymers. This communication summarizes certain novel reactions of pyrazine and its mono-, bi- and tetra-methyl substituted derivatives. We list in Table 1 some of the new complexes of univalent copper and of bivalent nickel and cobalt. Unfortunately, the low solubility of most of these compounds in suitable solvents renders inapplicable many physical techniques which are diagnostic of structure ; however, magnetic and spectroscopic data have proved specially valuable for this purpose.

Table 1

Metal ion	Complex	Physical form	C.N.*	Mag. mom. (B.M.)
Cu(I)†	(2 : 6-Di-Me-pyrazine)Cu_2Cl_2	Orange	2	0·0
	(2 . 5-Di-Me-pyrazine)Cu_2Br_2	Orange	2	0·0
	(Pyrazine)Cu_2I_2	Orange	2	0·0
	(Me-pyrazine)$Cu_2(CN)_2$	Yellow	2	0·0
	(Tetra-Me-pyrazine)$Cu_2(CN)_2$	Yellow	2	0·0
Ni(II)	(Me-pyrazine)$_4NiCl_2$	Pale green	6	3·31
	(2 : 6-Di-Me-pyrazine)$_2NiBr_2$	Yellow white	6	3·85
	(Me-pyrazine)$NiBr_2$	Green white	6	3·40
	(2 : 5-Di-Me-pyrazine)$NiBr_2$	Intense purple	4 ?	~0·0
	(2 5-Di-Me-pyrazine)$NiCl_2$	Green white‡	6	3·58
	(Pyrazine)$_2NiI_2$	Yellow	6 ?	3·23
Co(II)	(2 : 5-Di-Me-pyrazine)$CoCl_2$	Violet pink‡	6	5·53
	(2 : 5-Di-Me-pyrazine)$CoBr_2$	Dark blue§	4	4·68
	(Me-pyrazine)$CoBr_2$	Blue	4 ?	4·71
	(2 : 6-Di-Me-pyrazine)$_2CoI_2$	Deep green¶	4	4·76
	(2 : 6-Di-Me-pyrazine)$_2CoBr_2$	Violet pink	6	5·37

* C.N., Co-ordination number of metal ion.

† One of each type of complex only is quoted ; several others with the five ligands have been prepared.

‡ Isomorphous.

§ Dimeric in 2 : 5-dimethylpyrazine solution ; non-electrolyte in acetone.

¶ Monomeric in camphor ; non-electrolyte in acetone and nitromethane.

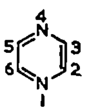

Fig. 1

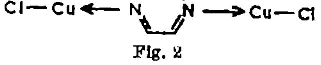

Fig. 2

Univalent copper gives rise to complexes in which there are *two* cuprous halide groups to each pyrazine molecule. The simplest structure for these complexes is shown in Fig. 2.

These represent the first complexes in which each nitrogen atom is bound to a metal atom. One is tempted to conclude that d_π–p_π bonding from the metal to the ligand could prove a means of increasing the negative charge on the ligand and hence of increasing the donor capacity of the second nitrogen atom. However, as will be discussed in a later communication, infra-red and other studies have led us to conclude that double bonding is probably less important than might have been expected at first sight.

Cupric complexes have been prepared, but these have not yet been studied in detail because their insolubility, coupled with a tendency to undergo reduction to the cuprous state, renders investigation of their structure rather difficult. Several different types of bivalent nickel compounds have been isolated. Complexes of the type ligand$_4$NiCl$_2$ are almost certainly six-covalent, and lose the ligand exceedingly readily, yielding complexes of the 1 : 1 type (see below). The 2 : 1 complexes (2 : 6-dimethylpyrazine)$_2$NiBr$_2$ and (pyrazine)$_2$NiI$_2$ achieve six-co-ordination through halogen bridging, and the 1 : 1 complexes (methylpyrazine)NiBr$_2$ and (2 : 5-dimethylpyrazine)NiCl$_2$ appear to reach the six-co-ordinate state by both halogen and pyrazine

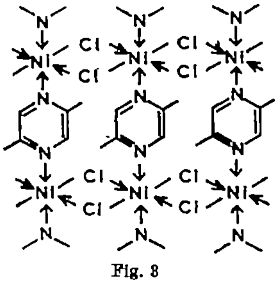

Fig. 3

bridging. A possible structure is illustrated in Fig. 3. These compounds are all paramagnetic with moments in the range expected for octahedral co-ordination (2·9–3·3 B.M.).

However, and in marked contrast, the purple (2 : 5-dimethylpyrazine)$NiBr_2$ is diamagnetic, a property generally associated (in the case of Ni(II) complexes) with a four-co-ordinate square planar or a six-co-ordinate tetragonal arrangement of the attached ligands. The behaviour is surprising in view of the fact that the corresponding chloro-derivative—this halide ion is usually assumed to give rise to a stronger ligand field than the bromide ion—is paramagnetic. Also the larger bromide ion is expected to cause greater steric interaction with the methyl group in a square complex than would occur in the chloro derivative. The chloro-complex is apparently octahedral six-co-ordinate, because it is isomorphous with the corresponding cobalt compound, the colour and magnetic properties of which strongly support octahedral co-ordination.

Of the bivalent cobalt compounds reported (2 : 6-dimethylpyrazine)$_2$$CoI_2$ is monomeric and its spectral and magnetic data point strongly to a tetrahedral arrangement. (2 : 5-dimethylpyrazine)-$CoBr_2$, on the other hand, is dimeric, though evidently still tetrahedral, and probably has a structure involving bromine bridges. The high magnetic moments, and colour, of the remaining cobalt complexes listed suggest strongly an octahedral structure. Those of the 1 : 1 type evidently involve both pyrazine and halogen bridging (as Fig. 3), whereas (2 : 6-dimethylpyrazine)$_2$$CoBr_2$ involves halogen bridging only.

Infra-red spectroscopy has proved to be a most useful tool in the elucidation of the structure of these complexes further data on which will be reported in detail shortly.

We are indebted to the Wyandotte Chemical Corporation, Detroit, Mich., for the supply of pyrazines and for financial support.

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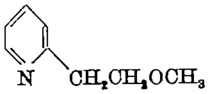
BIOCHEMISTRY

A New Anthelmintic with Unusual Properties

DURING studies on anthelmintic activity which have been in progress in these laboratories during the past few years, we have encountered an interesting degree of activity among a limited number of substituted pyridines. Nothing of practical value emerged from the early observations, but they caused us to retain an interest in related substances. Recently, through the courtesy of The Midland Tar Distillers, Ltd., we had the opportunity of examining some pyridine compounds made in their Research and Development Department, and one of them, prepared by Mr. P. Arnall, has proved of exceptional interest. This compound is 2-(β-methoxyethyl)-pyridine (U.K. Patent Application 40845/59; 40931/59; 20099/60):



It is a liquid and extremely soluble in water

Using experimental infections in mice with *Nematospiroides dubius*, *Nippostrongylus muris* and *Heterakis spumosa* and following methods developed by our colleague Dr. D. G. Davey, it has been possible to investigate the anthelmintic activity of this compound against nematodes inhabiting quite different regions of the alimentary canal. *N. dubius* inhabits the duodenum, *N. muris* the ileum and *H. spumosa* the colon, and marked activity has been obtained against all these species. In our experience a compound having similar activity against the three species is extremely rare, though we think this is not due to specific resistance on the part of the worms but to differences in their habitat. Thus the resistance of *N. dubius* to many anthelmintics appears to arise because it is confined to the duodenum.

The feature which makes 2-(β-methoxyethyl)-pyridine unique in our experience is the observation (Table 1) that it is more active when administered subcutaneously than orally. We know that the compound is excreted into the alimentary canal, seemingly along the whole length, and possibly this allows more intimate contact with all worm species whatever their location.

Table 1. COMPARATIVE ACTIVITY OF 2-(β-METHOXYETHYL)-PYRIDINE, PHENOTHIAZINE AND A MIXTURE OF BEPHENIUM EMBONATE AND HYDROXY NAPHTHOATE IN MICE

	2-(β-methoxyethyl)-pyridine		Mixture of bephenium embonate (1/3) and hydroxy naphthoate (2/3) (oral)	Phenothiazine (oral)
	Subcutaneous	Oral		
(1) *H. spumosa* Relative activity	360 1·0	560 0·64	1,054 0·34	800 0·45
(2) *N. muris* Relative activity	350 1·0	450 0·77	440 0·80	2,012 0·17
(3) *N. dubius* Relative activity	640 1·0	880 0·73	3,400 0·19	Approx. 14,000 0·05

The figures in the columns opposite the worm species give the dose in mgm./kgm. which is required for a 95 per cent reduction in worm burden.

Critical tests which will be reported in detail elsewhere have shown that 2-(β-methoxyethyl)-pyridine possesses greater activity against nematodes in mice than phenothiazine or a mixture of bephenium embonate (1/3) and hydroxy naphthoate (2/3), both of which are used in practice. Typical results are summarized in Table 1. Activity has also been demonstrated in mice against the immature forms of *N. dubius* and *N. muris*.

The results in mice with this compound were so promising that it was, and continues to be, tested in sheep, cattle and other domestic animals. Preliminary reports from various workers indicate that it may be of considerable value and, accordingly, it is being given more widespread use under the Imperial Chemical Industries brand name 'Promintic'.

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A Pyroninophilic Component in Pathological Serum Proteins and Urinary Proteins

Pyronine-methyl green is frequently used in histological investigations for staining ribonucleic acid and deoxyribonucleic acid[1].

Yeoman[2,3] applied this staining method to serum proteins and urinary proteins from patients with multiple myeloma. In each of the 11 cases examined, the abnormal protein fraction stained red-violet with pyronine-methyl green after paper electrophoresis of both serum and urine. This staining reaction was negative in 100 cases of hyperglobulinæmia and proteinuria. Yeoman presumes that the staining is specific for true myeloma proteins.

For some considerable time, investigations in our laboratory have been aimed at the chemical and immunological characterization of paraproteins. Consequently we have at our disposal a collection of sera from patients whose blood plasma contains one or several abnormal protein fractions. In addition, a few Bence Jones proteins are available. The specificity of the above-mentioned staining was tested in this material. A sample of each serum and each Bence Jones protein was submitted to electrophoresis at pH 8·6 in agar gel[4], and then stained with pyronine-methyl green in an acetate buffer at pH 4·8.

Table 1. Staining of Paraproteins with Pyronine–Methyl Green

	Positive	Negative
Multiple myeloma	9	—
Bence Jones proteins	8	—
Waldenstrom's macroglobulinæmia	13	19
Atypical macroglobulinæmia	4	3
Unexplained paraproteinæmia	1	7

The results obtained (see Table 1) show that the abnormal serum protein fraction stained with pyronine-methyl green in all cases of myeloma. Eight different isolated and purified Bence Jones proteins likewise produced a positive reaction.

A positive staining reaction was found in about half the cases of Waldenström's macroglobulinæmia and of so-called atypical macroglobulinæmia[5].

Sera from patients with an unexplained paraproteinæmia failed to stain, with one exception.

Therefore, this staining is unfortunately not specific for myeloma proteins from serum and urine, although these proteins showed a positive staining reaction both in the series examined by Yeoman and in our own material.

It is remarkable, however, that only abnormal protein fractions from serum and urine can be stained with pyronine-methyl green. This stainability might be taken to denote the presence of ribonucleic acid or deoxyribonucleic acid in these pathological protein fractions.

In order to verify this, the ultra-violet absorption spectrum of the electrophoresis-spots of these fractions was determined. This proved to be identical with that of proteins; ribonucleic acid or deoxyribonucleic acid was not demonstrable in these fractions (see Fig. 1).

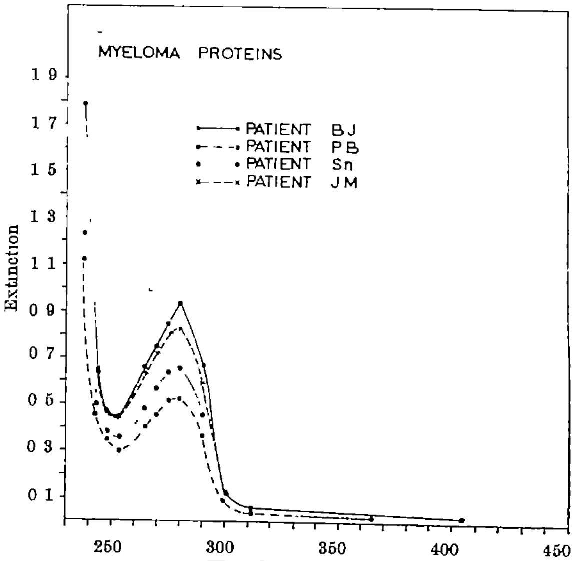

Fig. 1. Ultra-violet absorption spectrum of the pathological proteins in the serum of four myeloma patients

Both exposure to the influence of the enzyme ribonuclease (0·1 per cent in acetate buffer pH 6·0 at 37° C. for 30 min. and 7 hr., respectively) and treatment with 1 N perchloric acid at 4° C. failed to influence the stainability of the pathological protein fractions. The Feulgen reaction was also negative.

This warrants the conclusion that these proteins contain no ribonucleic acid or deoxyribonucleic acid.

Further investigations will be aimed at a definition of the component responsible for the positive staining reaction with pyronine-methyl green.

Thanks are due to Dr. D. Pragay for his kindness in supplying us with some Bence Jones proteins.

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Transferrin in Normal Cerebrospinal Fluid

Previous work has shown differences between some of the proteins in cerebrospinal fluid and those found in serum[1-3]. Thus paper-electrophoresis of concentrated normal cerebrospinal fluid may show a peak in the intermediate area between the beta- and gamma-fraction ('tau-fraction'), which is not seen in paper-electrophoresis of normal human serum[4-6]. The similarities and differences between transferrin in cerebrospinal fluid and in serum have been further investigated.

The cerebrospinal fluid was obtained by lumbar puncture from individuals without any neurological

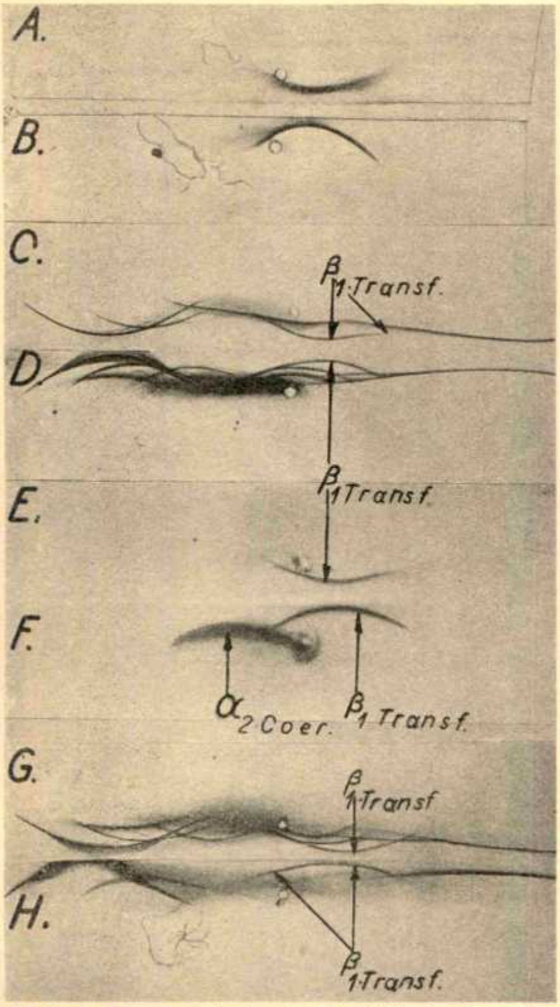

Fig. 1. *A*, Immuno-electrophoresis of iron-59 incubated cerebrospinal fluid, concentrated by pressure dialysis 50 times. The tracing is developed with a specific rabbit anti-transferrin antiserum. *B*, Immuno-electrophoresis of iron-59 incubated normal human serum, developed as *A*. *C* and *D*, The same as *A* and *B*, respectively, but now developed with a horse anti-human antiserum. *E* and *F*, The same as *C* and *D*, respectively, but now developed as autoradiogram. *G*, Immuno-electrophoresis of normal human serum developed as *C* and *D*. *H*, Immuno-electrophoresis of proteolytic digested normal human serum

disease. Care was taken to avoid any admixture of blood during the puncture. The fluid was concentrated by pressure dialysis and submitted to immuno-electrophoresis[3]. The development was performed with horse anti-human antiserum and specific rabbit anti-human transferrin, respectively.

A partial enzymatic digestion of normal serum proteins was achieved by means of pancreatic enzymes *NOVO*, possessing a proteolytic activity of 12 *NOVO* units/gm. = 600 Löhlein-Volhard units/gm.

The iron-binding capacity of transferrin and related proteins was demonstrated by autoradiography. 10–20 μl. of concentrated cerebrospinal fluid were incubated with about 10 μl. of a solution of radioactive iron-59 as ferric chloride; per sample 0·15 μc. was added with a specific activity of 5 mc./mgm. of iron. After incubation the serum was run immuno-electrophoretically and the plate exposed to a Kodak X-ray film.

The immuno-electrophoretic pattern of normal concentrated cerebrospinal fluid is shown in Fig. 1, and compared with that of normal human serum. The precipitation bow of transferrin from cerebrospinal fluid shows two differences from the corresponding bow of serum transferrin, indicating somewhat different physico-chemical properties of the two transferrins. The precipitation bow of cerebrospinal fluid transferrin has a double curvature and is more extended in the anode-cathode direction than the corresponding bow from serum. This extension is the result of a wider interval of mobility.

By incubation 2 ml. of human serum with 100 mgm. pancreatic enzymes *NOVO* at 37° C. for 1 hr. similar changes were found in the transferrin of this partially digested normal serum. Fig. 1 shows the immuno-electrophoretic tracing of such a proteolytically digested serum compared with that of genuine serum. No similar changes could be obtained from human sera treated with receptor-destroying enzyme (alias neuraminidase). In these experiments with the receptor-destroying enzyme several of the serum proteins showed a change in mobility, but not a reduplication of the immuno-electrophoretic bow. These changes in mobility are caused by a splitting off of the neuraminic acid[7].

The characteristic property of transferrin of binding iron was not qualitatively changed in cerebrospinal fluid. Fig. 1 shows the autoradiogram of an immuno-electrophoresis on concentrated cerebrospinal fluid incubated with iron-59. It is seen that the anodal as well as the cathodal part of the double-curved precipitation bow is able to bind iron. This figure, too, demonstrates the iron-binding capacity of normal human serum.

When the immuno-electrophoretic tracings of human serum are developed with a polyvalent horse anti-human antiserum not only transferrin but also coeruloplasmin was found to bind iron, and so was albumin when the maximal capacity of the two other proteins is surpassed (unpublished work).

In paper-electrophoresis on concentrated cerebrospinal fluid incubated with iron-59 the radioactivity could be traced to a maximum in the intermediate area between the beta- and gamma-fraction corresponding to the tau-fraction as seen by staining the paper for proteins with bromphenol blue.

No similar work on partial changes of the proteins compared with their normal carrier function is known to us.

One of us (J. C.) was in receipt of a grant from the National Multiple Sclerosis Society of Denmark.

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Methods for Determining the Rates of Catalase Synthesis and Destruction *in vivo*

Alterations in the amount of enzyme in a tissue can result from changes in the rate of synthesis or of destruction. The demonstration that 3-amino-1,2,4-triazole irreversibly inactivates catalase in the liver[1] without interfering with its resynthesis[2], and allyl-*iso*propyl acetamide blocks synthesis of new catalase[3] suggested that these compounds might be used as tools for studying the kinetics of catalase synthesis and destruction *in vivo*.

On injection of 3-amino-1,2,4-triazole, 1 gm./kgm. intraperitoneally, into rats there is a rapid fall in the liver catalase activity to a minimal value. After a lag period, during excretion of the aminotriazole, the catalase activity increases progressively over a period of 3–4 days, as originally described by Heim, Appleman and Pyfrom[4]. Experiments with radioiron have shown that this return of liver catalase activity results from the synthesis of new catalase[2]. Kinetic studies on the rate of catalase return following administration of aminotriazole show that the curve after the initial lag-period can be described by the equation:

$$C = \frac{k_S}{k_D}\left(1 - \exp(k_D t)\right)$$

where C is the catalase activity at any time, t, and where k_S is interpreted as the rate of catalase synthesis, and k_D as the fraction of catalase destroyed in unit time.

In order to check the validity of the above interpretation, k_D, the first-order rate constant for catalase destruction, was determined by blocking catalase synthesis with allyl*iso*propylacetamide (we are indebted to Dr. W. E. Scott of Hoffmann-LaRoche, Inc., Nutley, New Jersey, for a generous gift of this compound). For the experiment one group of animals was given a single injection of aminotriazole, 1 gm./kgm. intraperitoneally; a second group was given allyl*iso*propylacetamide 200 mgm./kgm. twice daily intraperitoneally; a third group was given both drugs, and the fourth was used for controls. Five animals from each group were killed daily and the liver catalase assayed spectrophotometrically[5]. The results are plotted in Fig. 1. It can be seen that while the catalase activity of the aminotriazole group was rising, that in the allyl*iso*propylacetamide group was falling. The group of animals given both drugs was of particular interest, since it showed whether or not catalase synthesis had actually been completely blocked by the allyl*iso*propylacetamide. An early increase in catalase activity was observed to a low plateau of about 10 per cent of the normal value. This represents catalase which can be destroyed by aminotriazole, but the synthesis of which is not blocked by the allyl*iso*propylacetamide. This catalase is presumably in a different location within the liver cell from most of the liver catalase, or else is in cells other than the hepatic cells of the liver. Since this catalase may have different rates of synthesis and destruction from the main catalase fraction, it may create minor deviations from the theoretical curve for the return of catalase activity following aminotriazole.

Liver catalase synthesis and destruction *in vivo*

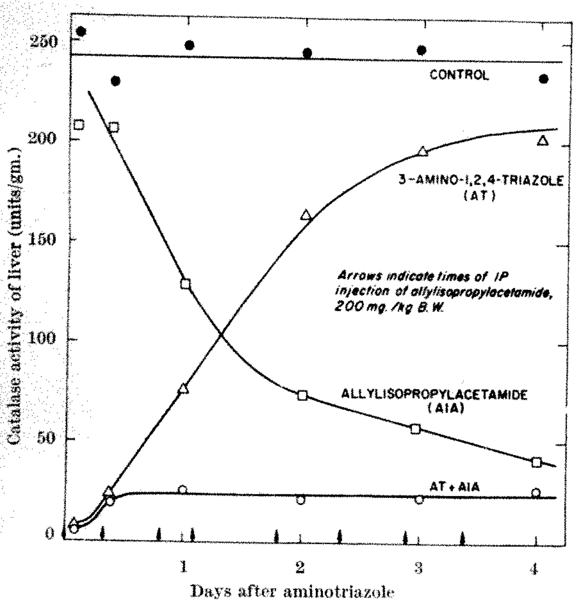

Fig. 1

Kinetics of liver catalase destruction *in vivo*

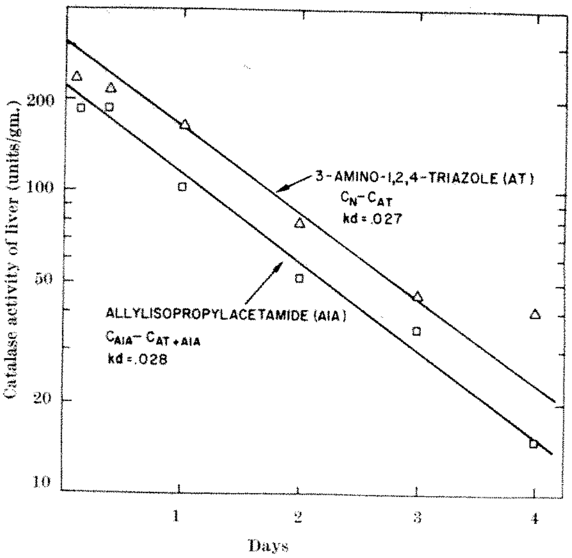

Fig. 2

If the rate at which the catalase activity of the aminotriazole group approaches the normal plateau is compared in a semilogarithmic plot (Fig. 2) with the rate at which the catalase activity of the allyl-*iso*propylacetamide group approaches its limit, which is the low plateau of the group given both drugs, it will be seen that two lines are obtained the slopes of which are nearly equal. The slope of these lines is equal to k_D, the rate constant for destruction. The rate constant for catalase destruction determined from the aminotriazole data is almost identical with that obtained when catalase synthesis is blocked with allyl*iso*propylacetamide. By substituting k_D in the equation: $k_S = k_D C_N$, where C_N is the catalase activity at the normal plateau, one can readily calculate k_S, the rate of catalase synthesis.

The above methods for studying the kinetics of catalase synthesis have the advantage that they are rather simple and require only injection of the drugs and assay of the liver homogenate for catalase activity. Only occasionally, to check the validity of a given system, does one need to use the radioisotopic methods, which require laborious purification of the enzyme to a constant specific activity. These techniques should prove useful in determining whether the lowering of catalase activity in the liver during starvation[6], during tumour growth[7], and following injection of toxohormone[8] results from a decreased rate of catalase synthesis or an increased rate of its destruction. Experiments along these lines are under way.

The above investigation illustrates the usefulness of inhibitors of enzymes and of enzyme synthesis for kinetic studies on the rate of enzyme synthesis and destruction. Many other enzyme systems have been described where similar types of inhibitors are available. As these systems are being studied, it may be useful to carry out kinetic studies similar to those presented here.

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Formation of Hippuric Acid from Phenylalanine labelled with Carbon-14 in Phenylketonuric Subjects

VARIOUS authors have recently demonstrated the formation of hippuric acid from endogenous benzoic acid in the mammalian organism. Fasting humans[1] as well as humans and rats on a lactalbumin–carbohydrate diet[2] excreted hippuric acid, and its formation in the intact rat could not be inhibited by sulphasuxidine[2] or chloramphenicol[3]. Application of deuterium and phenylalanine labelled with carbon-14 resulted in labelled urinary hippuric acid[2–4]. In addition, when benzoic acid was isolated from the urine of human or rats fed benzoic acid, labelled with deuterium or carbon-14, the specific activity was about 20 per cent lower, indicating dilution by endogenous benzoic acid[4,5]. During the investigation of phenylalanine turnover in human phenylketonuria[6], I had the opportunity of studying the formation of hippuric acid from phenylalanine. Phenylketonuric subjects are characterized by their inability to convert phenylalanine to tyrosine to any significant extent. This results in an accumulation of phenylalanine and its keto derivative, phenylpyruvic acid.

Two phenylketonuric patients were subjects for this work. Their plasma phenylalanine concentrations were dietetically adjusted to 17·0 (patient K. H.) and 47·0 (patient R. S.) mgm. per 100 ml. 20 μc. of uniformally labelled phenylalanine-^{14}C, corresponding to about $7 \cdot 7 \times 10^6$ c.p.m., were injected intravenously into both patients after an overnight fast. All urine was collected for 6 hr.

The total amount of the urinary hippuric acid was determined by the photometric method of Komei[7]; to avoid inhibition by urea, the urine was first treated with urease. A hippuric acid standard was run in urine with every sample. The urinary hippuric acid was isolated by adding 1·0 gm. of non-isotopic hippuric acid and 50 gm. of neutral ammonium sulphate to 100 ml. of urine and acidifying with concentrated hydrochloric acid to *p*H 2·0. The resulting crude precipitate was recrystallized by dissolving in sodium hydroxide and heating in a boiling bath for 1–2 min. after the addition of a small amount of 'Norit'. After filtering, the *p*H was again adjusted to 2·0 with dilute hydrochloric acid and the solution placed in ice-water. This step of recrystallization was repeated once more. The hippuric acid was then repeatedly recrystallized from hot water until the melting point (189–190° C.) and specific radioactivity remained constant. This end-point was achieved when no chlorine ions were demonstrable with silver nitrate in the supernatant.

Hippuric acid isolated from patient R. S. was hydrolysed by refluxing with 6 *N* hydrochloric acid for 90 min. The hydrolysate was extracted several times with one volume of ether. The ether extract was cautiously evaporated on a steam bath and the residue taken up in a small volume of alcohol. The alcoholic extract was diluted with 10 vol. of water, whereupon crystals appeared, and cooled in ice for about 1 hr. The benzoic acid so obtained was recrystallized from hot water until melting point (121° C.) and specific radioactivity remained constant. In addition a solution of 60 μgm. of the purified benzoic acid did not demonstrate any colour formation with ninhydrin on filter paper. A comparison of the specific radioactivity of hippuric acid and benzoic acid in this patient gave 797 and 801 c.p.m. per mmole respectively. This indicates that only the benzoic acid part derived from phenylalanine and that there is no appreciable amount of radioactivity in the glycine side-chain.

Table 1 summarizes the findings. The absolute turnover-rate of free phenylalanine was calculated from the disappearance of the radioactivity from the blood plasma. These experiments and their results will be described in detail elsewhere. The calculation of the fraction of phenylalanine converted to hippuric acid was made assuming that all urinary hippuric acid derived from phenylalanine. Therefore, these values are only an estimate of the upper limit of hippuric acid formation from phenylalanine in phenylketonuric subjects. These upper limits are likely to be considerably lower for normal individuals,

Table 1

Name	L-Phenylalanine-^{14}C injected (c.p.m.) (*A*)	Hippuric acid excreted (m*M* per 6 hr.) (*B*)	Specific activity hippuric acid (c.p.m. per m*M*) (*C*)	Absolute turnover-rate of phenylalanine (m*M* per 6 hr.) (*D*)	$\frac{B}{D} \times 100$
K. H.	$7 \cdot 60 \times 10^6$	0·76	$2 \cdot 78 \times 10^4$	20·9	3·6
R. S.	$7 \cdot 76 \times 10^6$	1·27	$2 \cdot 36 \times 10^4$	22·1	5·7

Absolute turnover-rate as defined in (*D*) is the ratio of the total amount of free phenylalanine present in the body to the turnover-time of free phenylalanine.

$\frac{B}{D} \times 100$ is the percentage of phenylalanine leaving the free phenylalanine pool which is converted to hippuric acid.

since they possess the ability to convert phenylalanine to tyrosine and have, consequently, a higher absolute turnover-rate than phenylketonuric patients.

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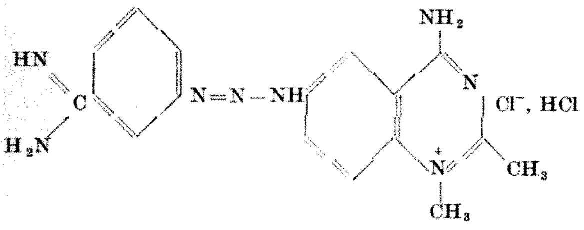
6-(*m*-Amidinophenyldiazoamino)-4-amino-1,2-dimethylquinazolinium Chloride Hydrochloride: a New Drug active against *Babesia canis*

As part of a study of diamidines which has extended over many years, we have recently screened for trypanocidal and babesicidal activity a number of compounds in which one of the amidine groups has been replaced by a cyclic amidine group. One of the most active of these is 6-(*m*-amidinophenyldiazoamino)-4-amino-1,2-dimethylquinazolinium chloride hydrochloride[1]:



(I)

(M. and B. 4986) (I). This compound, which contains the *m*-amidinophenyldiazoamino linkage present in the very active trypanocidal drug, *iso*metamidium[2], has babesicidal properties and is markedly active against *Babesia canis* in dogs.

The approximate maximum tolerated dose (subcutaneous) of M. and B. 4986 in healthy puppies was 25 mgm./kgm. body-weight, though marked tachycardia, accompanied by diarrhœa and vomiting, followed this dose, and persisted for about 1 hr. Fourteen days after a subcutaneous dose of 20 mgm./kgm. body-weight of M. and B. 4986 in adult dogs, there was no loss in weight, and no macroscopic signs of toxicity, apart from slight local skin thickening at the injection site, were observed on autopsy.

In therapeutic tests, a total of 46 puppies experimentally infected with *Babesia canis* were treated with doses of M. and B. 4986 between 0·5 and 10 mgm./kgm. body-weight. All the 12 infected puppies which received a subcutaneous dose of 2·5 mgm./kgm. survived, 3 were parasitologically negative within 24 hr., and all within 72 hr., 8 remained free of parasites for at least 30 days and 4 developed a parasitological relapse.

M. and B. 4986 (I) was prepared by coupling *m*-amidinobenzenediazonium chloride with 4,6-diamino-1,2-dimethylquinazolinium chloride. It was obtained as an orange crystalline solid, which decomposes at 237–238° C. (Found: N, 24·6; Cl, 15·3; H_2O, 11·4. $C_{17}H_{19}N_8^+,Cl^-,HCl,3H_2O$ requires N, 24·4; Cl, 15·4; H_2O, 11·7 per cent). It is approximately 0·5 per cent w/v soluble in water. In order to present a more soluble salt for field investigations the corresponding methanesulphonate (M. and B. 4986*A*) has been prepared as orange crystals, decomposing at 205–208° C. (Found: N, 20·6; S, 12·0; H_2O, 3·4. $C_{17}H_{19}N_8^+,CH_3SO_3^-,CH_3SO_3H,H_2O$ requires N, 20·6; S, 11·7; H_2O, 3·3 per cent); it is approximately 25 per cent w/v soluble in water.

Further details, including a report on the field investigations, will be published in due course.

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PHYSIOLOGY

Excretion of Tryptophan Metabolites after Physical Effort

IT has been shown that muscular effort is accompanied by the increased urinary excretion of one of the metabolites of tryptophan, namely, indoleacetic acid[1]. Pelikán and co-workers[2] have proved that there is increased excretion of another tryptophan metabolite, xanthurenic acid, after muscular effort.

Another tryptophan metabolite, 5-hydroxyindoleacetic acid, was then studied. The close relation between 5-hydroxytryptamine and the central nervous system is well known, and I wished to find out whether there is an increased urinary excretion of 5-hydroxyindoleacetic acid after muscular effort which is, moreover, often accompanied by considerable nervous tension.

5-Hydroxyindoleacetic acid is formed from 5-hydroxytryptamine by oxidase, and Sjoerdsma[3], Ressel[4], Udenfriend[5], de Gennes[6] and others state that its excretion is relatively constant. The normal urinary excretion of 5-hydroxyindoleacetic acid in men varies from 2 to 9 mgm./day[7]. Using a modification of Udenfriend's reaction, Schön and co-workers[8] recently found 1–6 mgm. (average 2·53 mgm./day). In the case of food only, the consumption of bananas results in increased excretion of this substance[9,10].

The urinary excretion of 5-hydroxyindoleacetic acid in men after various degrees of stress during athletics was followed. It was estimated in samples of urine within 30 min. before and after muscular effort, its determination being carried out by the method of Sjoerdsma, Weissbach and Udenfriend[3].

Altogether 50 men, aged 19–50, were examined after muscular effort lasting from 5 min. to 3 hr. Twelve men ran an average of 1,250 m. for 5 min. at maximum effort on a laboratory treadmill. Eight men were examined after a 3-km. cross-country race and 21 men after a marathon race. Also included in the group examined were 9 football players after a football match.

In no case was sulphosalicylic acid proteinuria found before the effort. With the exception of 4 well-trained cross-country runners and 5 marathon runners, proteinuria could always be detected after the effort was made (ranging from slight turbidness to heavy protein precipitation). After a marathon race, proteinuria of 6–166 mgm./100 ml. (average value 47·1 mgm./100 ml.) was detected by means of Exton's method.

In no case, either before or after the effort, could any 5-hydroxyindoleacetic acid be detected. From this it can be concluded that, even when accompanied by considerable nervous excitement, muscular effort does not result in increased urinary excretion of this substance.

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Anionic Permeability of the Inhibitory Postsynaptic Membrane of Motoneurones

It has been postulated that the inhibitory postsynaptic potential in motoneurones is caused by the movement of ions across patches of the postsynaptic membrane that are momentarily (1–2 msec.) made very permeable for ions less than a certain critical size[1]. A sufficiently increased intracellular concentration of anions capable of passing through the postsynaptic membrane would change the normally hyperpolarizing inhibitory postsynaptic potential into a depolarizing one. In accordance with the hypothesis it was shown that injections of the small ions, Br^-, Cl^-, NO_3^-, and SCN^-, but not injections of the large HCO_3^-, $CH_3CO_2^-$, SO_4^{--}, $H_2PO_4^-$ or HPO_4^{--}, were effective in transforming the inhibitory postsynaptic potential into a depolarizing response. The diameter of the largest effective anion (derived from limiting ion conductances[2] and expressed relative to $K^+ = 1{\cdot}00$) was 1·11 and that of the smallest not effective, 1·65 (ref. 1). A weakness in the experimental evidence was the relatively few ions tested and the gap in size between the effective, and not effective, ions. Furthermore, it was not possible to exclude a slight effect of the bicarbonate ion (diameter 1·65) (ref. 1).

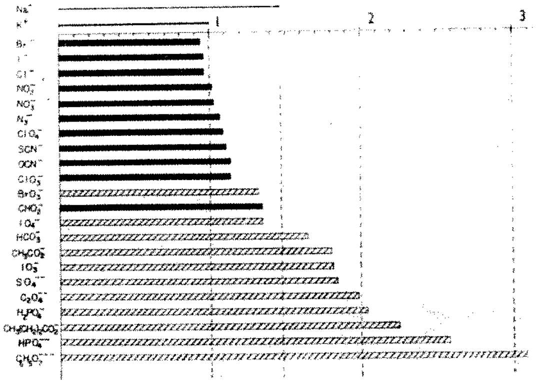

Fig. 1. The ions specified in the left column are arranged in order of hydrated ion size (derived from limiting ion conductances (ref. 2) and expressed relative to $K^+ = 1{\cdot}00$). The black bands indicate ions that are effective in changing the inhibitory postsynaptic potential into a depolarizing response; the hatched, those which are not effective. It should be noted that these results are consistent with the hypothesis that potassium ions can pass through the membrane but not sodium ions (ref. 1)

We have now extended the previous investigation and tested 22 different species of anions. Microelectrodes containing the anion to be tested were inserted into motoneurones and used both for injection and recording. Most tests were made on knee flexor motoneurones that were inhibited by quadriceps group Ia afferents. The anions were injected by a steady current of 3–10 × 10^{-8} amp. during 1 min. or more. The effect of the injection was judged by the polarity and size of the inhibitory postsynaptic potential immediately following the injection. The effects of the injections were always reversible within a few minutes after the injection. This recovery is attributable to the outward diffusion of the injected ions across the cell membrane.

The results are illustrated by the histogram (Fig. 1) showing the ions arranged according to their diameters. It appears that all ions with a diameter less than 1·32 (bromate) were effective in producing the reversible change of the inhibitory postsynaptic potential into a depolarizing response. Larger ions were ineffective except for the only slightly larger formate-ion (1·35). Injections of bicarbonate ions or carbonate ions never resulted in an immediate change of the inhibitory postsynaptic potential. After several injections of any of these ions there was often a gradual decrease of the hyperpolarization and finally a change to a depolarizing inhibitory postsynaptic potential. This late cumulative change is presumably due to secondary effects of these ions and cannot be taken as evidence for a similar action as that exerted by the small ions. Hence our findings support the hypothesis that the activated inhibitory postsynaptic membrane has an increased permeability for all anions less than a certain critical size.

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Lack of Uptake of Catecholamines after Chronic Denervation of Sympathetic Nerves

DENERVATION of sympathetically innervated structures has been known to cause supersensitivity of these organs to catecholamines[1] and depletion of these hormones[2]. However, no conclusive explanation has been offered for these findings. To clarify these phenomena further we compared the uptake of injected DL β-^{3}H-noradrenaline and DL β-^{3}H-adrenaline by intact and denervated tissue.

The right superior cervical ganglion was removed from cats, and sufficient time (more than 5 days) was allowed to elapse to ensure degeneration of the nerve fibres. The operated cats all had pupillary contraction and relaxation of the nictitating membrane on the denervated side. Tritium-labelled noradrenaline or tritium-labelled adrenaline was administered intravenously, 1 hr. later the animals were killed and the ^{3}H-catecholamine content was examined[3,4] in structures innervated by the cervical sympathetic nerve.

A striking reduction in the levels of ^{3}H-noradrenaline was observed in the chronically denervated structures (Table 1). Similar results were obtained after administration of ^{3}H-adrenaline. No differences were found in cats in which the right cervical ganglion was removed 15 min. before the administration of ^{3}H-noradrenaline. It is apparent from these results that the nerve endings or neighbouring structures take up and retain circulating catecholamines.

Table 1. EFFECT OF DENERVATION ON THE UPTAKE OF ^{3}H-NORADRENALINE AND ^{3}H-ADRENALINE

Tissue	Chronic denervation				Acute denervation	
	Noradrenaline		Adrenaline		Noradrenaline	
	Denervated	Innervated	Denervated	Innervated	Denervated	Innervated
External ocular muscle	5·9	47·9	1·8	15·4	24·9	26·6
Retractor bulbi muscle	1·9	11·3	1·8	8·2	13·2	13·4
Lachrymal gland	3·2	44·7	3·0	24·7	—	—
Salivary gland	5·7	42·4	2·5	115·8	75·5	88·6

In the chronic experiment the right superior cervical ganglia were removed from six adult male cats under pentobarbitone anæsthesia. After 5–21 days, 4 cats were given ^{3}H-noradrenaline and 2 cats ^{3}H-adrenaline (25 μgm./kgm. DL base, specific activity 3·25 mc./mgm.) intravenously under pentobarbitone anæsthesia. One hr. after the administration of the catecholamines the cats were decapitated and the ^{3}H-catecholamine content was assayed in denervated and innervated structures. In the acute experiment the right superior cervical ganglion from 2 cats was removed 15 min. prior to the administration of ^{3}H-noradrenaline. Results are expressed in mμgm. ^{3}H-noradrenaline or ^{3}H-adrenaline per gm. of tissue.

We have previously shown that binding is an important mechanism of catecholamine inactivation[3]. The present findings indicate that degeneration of nerve endings results in the inability to bind these hormones and consequently this mechanism of inactivation is inoperative. The effective concentration of unbound, active catecholamines in the vicinity of the receptors in denervated tissue would be expected to persist for longer periods, thus resulting in supersensitivity. Cocaine, which reduces the uptake of circulating catecholamines by the tissues[5], presumably by interfering with binding, also causes supersensitivity.

Removal of free catecholamines can also occur by enzymatic transformation. Catechol-O-methyl transferase[6] and monoaminoxidase[7] have both been considered to be involved in the metabolic inactivation of the catechol hormones. However, Burn *et al.*[8] have shown that inhibition of monoaminoxidase by iproniazid does not prolong the contraction of the nictitating membrane after stimulation of the sympathetic ganglion, whereas Wylie *et al.*[9] have demonstrated that inhibition of catechol-O-methyl transferase by pyrogallol prolongs the contraction.

From the results described here and elsewhere[3], inactivation of catecholamines at nerve endings and tissues is a complex process involving enzymatic transformation and binding. It appears that binding occurs primarily at the nerve endings and enzymatic transformation (presumably by catechol-O-methyl transferase) in tissue and at receptor sites.

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Effects of Pantothenic Acid (Calcium Pantothenate) and of Thyroxine on the Development of Chicken Embryos

IN earlier investigations, we have demonstrated[1] that the injection of 100 μgm. of calcium pantothenate into the yolk sac of chicken eggs after incubation periods of 4 and 10 days is responsible for an increase in body-weight, lasting 3–6 days. Together with the increase in body-weight, we have observed also an increase in tissue differentiation (studied by means of serial histological examination) especially of the nervous system (ganglia), kidneys, glandular stomach, liver and cartilages.

In the present work, eggs of White Leghorn–New Hampshire fowls, divided into four lots, were used. In the first lot 100 μgm. calcium pantothenate in sterile physiological solution (total volume of 0·1 ml.) was injected into the yolk sac after 4 and 10 days incubation. The second lot was injected with 5 μgm. thyroxine (Roche); the third with 100 μgm. of calcium pantothenate together with 5 μgm. thyroxine. The fourth lot (kept as control) was injected with 0·1 ml. of sterile physiological saline.

In six eggs of each group, embryonal life was interrupted after 7, 10, 13, 16, 19, 21 days of incubation for the eggs injected after 4 days incubation.

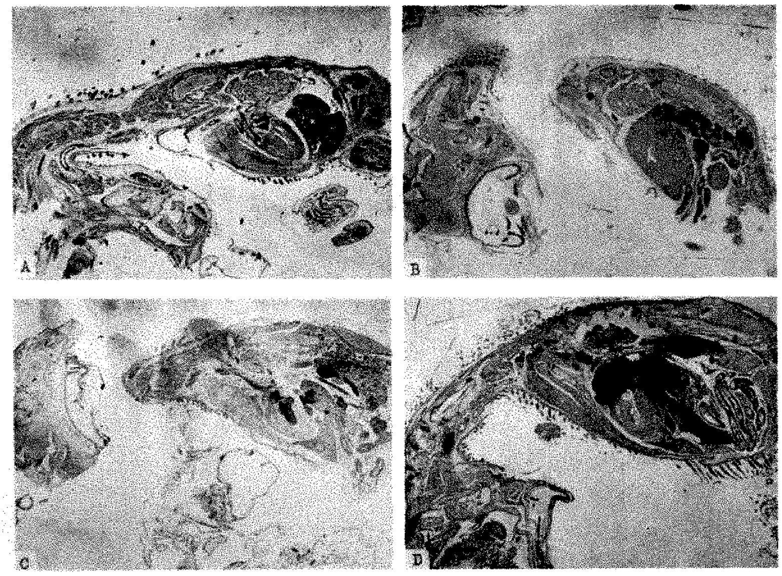

Fig. 1. Chicken embryos, tenth day of incubation, injected on fourth day. *A*, Control ; *B*, thyroxine ; *C*, calcium pantothenate ; *D*, calcium pantothenate + thyroxine

For the eggs injected after 10 days incubation, embryonal life was interrupted after 13, 16, 19, 21 days of incubation.

All the embryos treated survived until the end of incubation. The embryos were weighed with all fœtal membranes after remaining in a refrigerator at 4° C. for 6 hr.

Our results may be summarized as follows : (1) Embryos treated with only 100 μgm. of calcium pantothenate show an increase in body-weight, which is more marked in the embryos injected after 10 days incubation, and lasting 5–6 days, as was demonstrated in our earlier work[1]. (2) Embryos treated only with a dose of 5 μgm. of thyroxine show, on the contrary, a reduction of body-weight, lasting from the day of injection until almost the end of embryonal life. (3) Embryos treated with calcium pantothenate together with thyroxine show a tremendous increase of body-weight compared to the controls, and still more striking if compared to the embryos treated with thyroxine alone. They are also bigger than the embryos treated with calcium pantothenate alone. This increase lasts until almost the end of embryonal life, for a longer period than that observed in the embryos treated with calcium pantothenate alone.

In Fig. 1 are shown examples of histological sections of 10-day embryos that give an idea of the effects of calcium pantothenate, thyroxine and thyroxine + calcium pantothenate on the body and on the tissues of the embryos treated.

The histological serial slides (which will be described in detail elsewhere) show clearly, in the embryos treated with calcium pantothenate alone and with thyroxine alone, a major, marked, differentiation of organic tissues, particularly evident in liver, nervous system, glandular stomach and cartilages. This increase in tissue differentiation is also very clear, and may be more marked, in the embryos treated with calcium pantothenate together with thyroxine.

In the light of these experimental results, it is possible to state : (*a*) that the pantothenic acid (calcium pantothenate) and thyroxine mutually increase, if injected together, their separate effects on organic tissue differentiation of developing chicken embryos ; (*b*) that pantothenic acid prevents the well-known reduction of body-weight produced by thyroxine in chicken embryos ; (*c*) on the other hand, thyroxine enhances the increase in body-weight produced by calcium pantothenate when that substance is injected alone.

The effects observed may be explained by the marked influence that pantothenic acid exerts on the intermediate metabolism, and particularly on protein synthesis, as a constituent of co-enzyme A.

It seemed of interest, therefore, to recall that de Franciscis and Landauer[2] have recently demonstrated in chicken embryos, of almost the same age as those we used, that cortisone and insulin exert different effects on the development of the embryos (micromielia, abnormalities, body-weight, etc.), when the two hormones are injected together or injected alone.

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Experimental Pre-Thrombotic State of an Organism resulting from the Dysfunction of the Physiological Anticoagulating System induced by an Atherogenic Diet

We have already shown[1–6] the existence in the animal organism of a reflex-humoral protective system which prevents the coagulation of blood circulating in the vascular system. In connexion with these results we became interested in the work of Wilgram[7,8] as well as in that of Thomas and Hartroft[9]. These authors have observed spontaneous myocardial infarctions in rats kept for several months on a diet enriched with fat and cholesterol. We supposed the diet indicated to produce a negative influence on the functional condition of the physiological anticoagulating system, due to which fact a pre-thrombotic state is seen to arise in the animal organism.

To verify this supposition, 100 adult male rats were transferred from a natural diet to that of Wilgram[7,8], supplemented with vitamin E in the form of an oily preparation from natural sources. Rats of the same age receiving a natural laboratory diet served as control. The functional state of the anticoagulating system in test and control rats was checked at various experimental stages by injecting thrombin or thromboplastin solution (preparation from the rat brain) into the jugular vein. The experiment proved that the fat-cholesterol diet began to produce a negative effect on the functional state of the anticoagulating system 1½–2 months after its application, and in five and more months it blocked the activity of that system. Intravenous administration of moderate thrombin doses to such test animals causes their immediate death following intravascular clotting, in contrast with the control remaining alive after the same thrombin doses injected intravenously (Table 1). In all rats fed the experimental diet for five and more months the following changes were established: a rise in the concentration of fibrinogen (exp., 433 mgm. per cent; control, 312 mgm. per cent), a decrease of the blood fibrinolytic activity (exp. 7·5 per cent; control, 48·0 per cent), a sharp increase of the blood plasma tolerance to heparin (exp., 2 min. 25 sec.–6 min. 28 sec.; control, 7 min. 31 sec.–22 min. 48 sec.), and a ten-fold increase of the total cholesterol content in the blood (exp., 943 mgm. per cent; control, 95 mgm. per cent). It seems that a combination of the first three symptoms may be used to diagnose a pre-thrombotic state of the organism.

Table 1. DEPRESSION OF THE FUNCTION OF THE PHYSIOLOGICAL ANTICOAGULATING SYSTEM IN ANIMALS KEPT ON A PROLONGED FAT–CHOLESTEROL DIET (AVERAGE RESULTS)

Experimental conditions	Animal body-weight (gm.)	Thrombin dose* intravenously (ml.)	No. of animals		
			Total in the experiment	Survived	Died of thrombosis
Experiment: Rats kept on a fatty diet from 5 months to 7 months and 15 days	221–276	1·0–1·2	25	3	22
Control 1: Rats kept on a natural laboratory diet	223–276	1·0–1·2	25	22	3
Control 2: Rats kept on a fatty diet for 5 months. Thrombin inactivated by heating	251	1·7 inactivated	12	12	0

* Thrombin solution clotted an equal volume of oxalated blood in 5 sec. at 37°.

As a prophylactic against the formation of thrombi in test animals in a pre-thrombotic state a soybean trypsin inhibitor was used, as suggested by the results previously obtained[10]. The inhibitor preparation was isolated according to Künitz's method[11].

According to the results in Table 2 the trypsin inhibitor injected intravenously 5 min. before the thromboplastin injection has shown a good prophylactic action against thrombus formation.

Table 2. PROTECTIVE ACTION OF TRYPSIN INHIBITOR AGAINST THE FORMATION OF THROMBI IN THE CASE OF INTRAVASCULAR INJECTIONS OF LETHAL THROMBOPLASTIN DOSES INTO RATS KEPT ON A FAT–CHOLESTEROL DIET FOR 5 MONTHS (AVERAGE RESULTS)

Dose of 5 per cent trypsin inhibitor solution intravenously (ml.)	Dose of thromboplastin (ml.)	Average animal weight (gm.)	No. of animals		
			Total in experiment	Survived	Died of thrombosis
Experiment (a fatty diet)					
1·4	2·1	254	10	10	0
Control (a fatty diet)					
0	2·1	253	10	0	10

The experimental results obtained by us indicate that the development of the myocardial infarction in rats fed the atherogenic diet of Wilgram[7,8] and of Thomas and Hartroft[9] is connected with the appearance in the test animal of a pre-thrombotic state resulting from a depression of the function of the physiological anticoagulating system.

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HÆMATOLOGY

Haptoglobin and Transferrin Types in the Kuru Region of Australian New Guinea

KURU is a rapidly progressive and fatal neurological disease found to occur with high frequency among the Fore natives in the Eastern Highlands of New Guinea. Their tribal area, of several hundred square miles, is in a remote and rugged part of the highlands which has only recently become accessible to Europeans. Between 20,000 and 30,000 people live in the area affected and, of these, about 1 per cent is afflicted with the disease at any one time. Altogether kuru is responsible for more than half the deaths of Fore females and about 10 per cent of males. Extensive studies have as yet failed to reveal evidence of any environmental or nutritional factor involved in the production of kuru[1]. The possibility of the Fore people having a genetical predisposition to kuru was first mentioned in 1957[2]. Shortly afterwards, when a preliminary genetical study had been made, distinctive family patterns were found[3,4], and it was then suggested not only that the disease had an important familial component but also that this was quite possibly based on a single gene locus. The genetical hypothesis, advanced tentatively, was that kuru is under the control of autosomal alleles *Ku* and *ku* such that in females the homozygotes *KuKu* and heterozygotes *Kuku* are early- and late-onset victims, respectively, while in males the homozygotes *KuKu* are kuru victims but the heterozygotes are clinically normal. There are obvious weaknesses in basing inferences on genealogical data obtained from such primitive people through pidgin-English-speaking native interpreters. At the present time, however, this is the only way in which such material can be obtained. Nevertheless, it must be noted that the genealogical data gathered from many different parts of the affected region in extensive field studies during the past two years all exhibit essentially the same distinctive family patterns[5].

A puzzling observation has been Curtain's[6] reported association of kuru with the haptoglobin gene Hp^2. Using starch-gel electrophoresis, he found that the frequency of the Hp^2 gene in 54 kuru patients was 61 per cent as against only 36 per cent in 62 'normal' Fore individuals. Because this statistically significant difference seemed hard to reconcile with the genetical interpretation for kuru, an independent test for this association has been carried out. Blood samples were obtained by venepuncture from kuru patients and also from healthy Fore natives who were the parents of young female and male victims of kuru. Such parents, though not having any of the clinical symptoms of kuru, would be genetic carriers of the disease according to the above hypothesis. The samples were collected into Bayer venules and flown to Australia in ice-cold flasks. Haptoglobin typing of the sera was carried out in Perth using the vertical method of starch-gel electrophoresis as described by Smithies[7]. The results obtained are shown in Table 1.

Table 1. HAPTOGLOBIN GROUP FREQUENCIES

	No. tested	Haptoglobin group			Gene frequency	
		1—1	1—2	2—2	Hp^1	Hp^2
Kuru patients	47	21	20	6	0·66	0·34
Others	35	16	15	4	0·67	0·33
Total	82	37	35	10	0·66	0·34

The chief point which emerges from the present work is that there is no indication of any difference between the frequencies of the haptoglobin types in kuru patients and in those other Fore natives chosen for comparison. Furthermore, the frequencies of the Hp^1 and Hp^2 genes in both these groups are very similar to those reported by Curtain, not for his kuru patients, but for his control group of 'normal' individuals.

Several points of interest arise when the present results are compared with those reported for other populations. In this material, there is no Hp 0–0 individual nor is there anyone of the modified Hp 2–1 phenotype. These two types, which seem to be common in African Negroes[8], are rare in Europeans and in Japanese[9], Indian, Chinese and Malayan samples[10]. Neither type has been found in 89 Australian aborigines from South Australia, and there was only one 0–0 in a sample of 131 aborigines from the Western Desert (unpublished observations). The high frequency of the Hp^1 gene in this material is of interest. It is slightly higher than the frequency of 0·63 which has been reported for Micronesians of the Marshall Islands[11], and it is close to the highest value yet reported, namely, 0·72 for African Negroes. These values are much higher than in any of the series of South East Asian peoples so far tested[10], and they are in marked contrast to the low value of about 0·13 for the Hp^1 gene frequency found in Australian aborigines from South and West Australia—although a frequency of 0·64 for Hp^1 has been reported by Budtz-Olsen[12] for 100 aborigines from Central Australia, based on determinations using electrophoresis on paper.

The frequencies of the transferrin differences, typed simultaneously with the haptoglobins during starch-gel electrophoresis, are shown in Table 2. It is of interest that the frequency of the TfD_1 variant is the highest that has yet been reported, in published work, for any population. However, recent unpublished work in our laboratories indicates that it has an even higher frequency in Australian aborigines and that it is common also in the Veddahs of Ceylon.

Table 2. TRANSFERRIN GROUP FREQUENCIES

	No. tested	Transferrin group			Gene frequency	
		CC	CD_1	D_1D_1	C	D_1
Kuru patients	45	39	5	1	0·92	0·08
Others	35	26	8	1	0·86	0·14
Total	80	65	13	2	0·89	0·11

For both the haptoglobin and transferrin types, the observed genotypic frequencies shown in Tables 1 and 2 are in close agreement with those expected with random mating.

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Hæmoglobin E in Egypt

HÆMOGLOBIN E is generally thought to occur exclusively among people of Mongoloid origin ; the greatest incidence being in the Burmese[1], Siamese[2], Cambodians[3], Indonesians[4] and Malayans[5]. The occurrence of hæmoglobin E in other peoples, for example, the Eti-Turks, an Arabic-speaking group living in southern Turkey[6], is usually accompanied by definite historic or familial ties with the South Eastern Asian. The discovery of hæmoglobin E in Egypt among a family which, so far as could be determined, has no direct relationship to the Asiatic (Mongoloid) is now reported.

The propositus was a 39-year-old male. The faces of the subject and two of his brothers and a niece who were seen are not those of the typical Arab. They represent, however, the features encountered among average Egyptians. Histories taken from the parents of the propositus revealed that the family had resided in the Nile Valley (delta) for many generations and had always gained a living as agricultural workers (fellaheen).

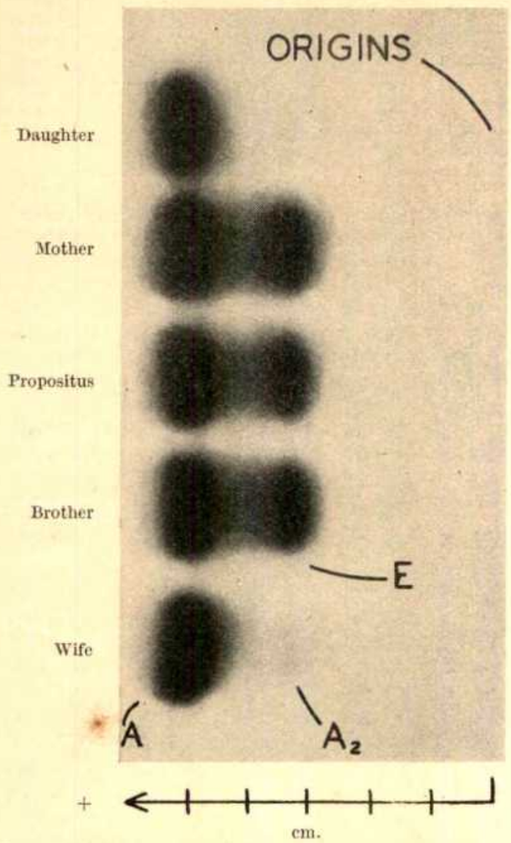

Fig. 1. Photograph of an unstained starch-gel slab after electrophoresis of hæmolysates from members of a family having the hæmoglobin E trait. Electrophoresis at 7 V. per cm. for 16 hr. Non-continuous buffer system (*tris*-maleate, 0·07 *M*, *p*H 8·7, for gel mixing; and borate, 0·3 *M*, *p*H 8·7, for bridge solution)

Twelve members of the family were examined. Nine were detected by starch-gel electrophoresis[7] to be hæmoglobin E heterozygotes. Consistent with previously reported results[8], we found no hæmatological or radiographical evidence of hæmolytic anæmia. A photograph of a starch-gel slab in which the hæmolysates of the propositus, his mother, wife, child and brother were submitted to electrophoresis is shown in Fig. 1. Of the family members studied, only the wife of the propositus (a second cousin on the maternal side), her brother and a daughter were free of hæmoglobin E. The concentration of the abnormal pigment was greater than 29 per cent in all cases.

It is interesting to speculate on the origin of hæmoglobin E in Egypt.

(1) *Origin by mutation.* Although it is difficult to establish unequivocally, the probability of gene mutation must be considered in distal occurrences of abnormal hæmoglobins. In this instance, we have demonstrated hæmoglobin E in persons who reside at great distances from its assumed focus and who are considered of a different race.

(2) *Origin as a new hæmoglobin.* Hæmoglobin E in the Asiatic and that reported here might arise from independent genetic mechanisms. Although standard identification tests did not vary from previously published results, we were able to derive some differences in migration-rates between hæmoglobin E and A_2, when the two were subjected to starch-gel electrophoresis in the second dimension. This is being studied further.

(3) *Origin by racial ties with Mongoloids.* This need scarcely be considered in the light of known anthropological differences between the two groups.

(4) *Penetration of hæmoglobin E gene.* This is the most attractive possibility, provided gene mutation is not the prime factor. It is indeed reasonable to harmonize the presence of hæmoglobin E in people of the Middle East with its existence among the Mongoloids of south Asia. It is in these areas and among these people that Islam was established in the Far East. Brumpt *et al.*[3] direct attention to the fact that in Cambodia there was something more than a coincidental increase of hæmoglobin E among natives of the Muslim faith. Another factor, of course, would be the Mongol invasions of the Middle East in A.D. 1258. Their attack extended as far as the eastern border of Sinai. They dominated the area of Syria and Iraq for decades. Most probably it was during one of these historical events that the gene of hæmoglobin E passed into the family we have examined.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the U.S. naval service at large.

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PATHOLOGY

Immunological Cross-Reactions between Salk 'Altered' Monkey Heart Cells and Ehrlich Ascites Carcinoma Cells

RECENT work[1] has shown that the metabolism of Ehrlich ascites carcinoma cells (EA cells) can be inhibited by small quantities of specific antiserum in the presence of complement. We have now observed that antisera prepared against the Salk 'altered' monkey heart cells (MHS cells)[2] show activity not only against the homologous cells, but also against Ehrlich ascites carcinoma cells and HeLa cells as well.

Antisera were prepared in rabbits as described in a previous communication[1]. The sera were lyophilized immediately after preparation and used without inactivation, unless otherwise specified. The methods of incubation and analysis were identical to those described previously[1].

Table 1. EFFECT OF ANTISERA ON DIFFERENT CELL LINES

Cells	Additions (antisera)	Uptake (c./min./mgm. dry weight)	Incorporation (c./min./mgm. protein)
MHS	Nil	1,360	100
	MHSAS	710	68
	EAAS	1,320	106
EA	Nil	3,140	144
	MHSAS	140	69
	EAAS	176	25
HeLa	Nil	2,060	46
	MHSAS	147	3
	EAAS	1,750	36

Glycine 2 μM, at 45 × 10^3 c.p.m./ml. Aerobic incubation for 1 hr. in Krebs–Ringer phosphate. Gas phase, oxygen.

Table 2. EFFECT OF DIFFERENT SOURCES OF COMPLEMENT, USING SALK 'ALTERED' MONKEY HEART CELL ANTISERUM AND EHRLICH ASCITES CARCINOMA CELLS

Additions	Uptake (c./min./mgm. dry weight)
Nil	2,200
Normal fresh rabbit serum	2,200
Normal fresh guinea pig serum	2,040
MHSAS inactivated	1,890
MHSAS inactivated + fresh guinea pig serum 1 : 1	1,790
MHSAS inactivated + fresh rabbit serum 1 : 1	1,100
Lyophilized MHSAS	420

Conditions as in Table 1.

Table 3. EFFECT OF THE ALTERATION OF THE CELL-SERUM RATIO

MHS cells (mgm. dry weight)	MHSAS (ml.)	Incorporation (per cent inhibition)	Uptake (per cent inhibition)
5·7	0·2	79	62
	0·1	37	17
	0·05	0	10
2·85	0·2	97	88
	0·1	54	43
	0·05	29	0
1·9	0·2	100	93
	0·1	75	75
	0·05	31	0

Conditions as in Table 1.

Conditions of the experiments were as follows: The incubations were carried out in Krebs–Ringer–bicarbonate medium (Tables 1 and 3) or Krebs–Ringer–phosphate medium (Table 2) under aerobic conditions for 1 hr.

The constituents per 1 ml. were :

Test serum	0·1 ml.
Complement (where applicable)	0·1 ml.
Glycine-1-^{14}C, 45 × 10^3 c.p.m.	2 μmoles
Glucose	10 μmoles
Cell suspension	0·5 ml.

The dry weight of the cell suspension was 4·0 mgm. unless otherwise indicated.

The results are expressed as follows : (*a*) uptake : counts/min./mgm. dry weight of cells ; (*b*) incorporation : counts/min./mgm. protein.

The results presented in Table 1 show that, while Salk 'altered' monkey heart cell antiserum markedly affects the uptake and incorporation of glycine-1-^{14}C by the Ehrlich ascites carcinoma cells and HeLa cells as well as by the Salk 'altered' monkey heart cells, the Ehrlich ascites carcinoma cell antiserum (EAAS) is much less effective against the heterologous cells.

We have also observed, as indicated in the previous report[1], that the reaction is dependent on the presence of complement. Inactivation of the antiserum at 56° C. for 30 min. brings about a loss of inhibitory activity. The conventional source of complement, namely, guinea pig serum, restores little of the inhibitory activity. Normal fresh rabbit serum is much more effective, but unheated (lyophilized) antiserum is always the most effective inhibitor. Table 2 shows the results of a typical experiment illustrating this phenomenon.

Since it was known from previous experiments (unpublished results) that with the Ehrlich ascites carcinoma cells and the homologous antiserum there is a ratio of cell to antiserum at which maximal inhibitory activity is observed, the effects of changing the cell/antiserum ratio with the Salk 'altered' monkey heart cells were considered.

The results in Table 3 indicate that the inhibition observed at a given concentration of serum is much greater when few cells are used.

Recently, Defendi and Colter[3] observed immunological cross-reactions between different cell lines. Our results, using different techniques, indicate that such cross-reactions exist between Ehrlich ascites carcinoma cells, Salk 'altered' monkey heart cells, and HeLa cells ; thus antisera against the Salk 'altered' monkey heart cells also inhibit the metabolic activity of the Ehrlich ascites carcinoma cells and HeLa cells. However, antiserum against Ehrlich ascites carcinoma cells does not affect the Salk 'altered' monkey heart cells to the same extent.

Further experiments are required using cross-absorption techniques to decide which cell fractions are responsible for this phenomenon.

We are indebted to the Connaught Medical Research Laboratories, Toronto, for having made the Salk 'altered' monkey heart cells available to us. This work was supported by a grant from the National Cancer Institute of Canada and the National Research Council of Canada. We are grateful for the help and encouragement given by Profs. J. H. Quastel and R. W. Reed.

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Inhibition of Experimental Cirrhosis by Carbon Tetrachloride following Treatment with Aminoacetonitrile

EXPERIMENTS on lathyrogenic compounds have shown that they exert a delaying action on collagen formation[1–4]. The mechanism by which they act is still unknown.

Owing to the biological interest of these findings, and in view of their possible pharmacological implications in those pathological conditions where the new formation of collagen is the fundamental lesion or a most important component of the pathological picture, the present experiments were carried out in order to study the action of aminoacetonitrile (AAN), one of the lathyrogenic agents, on experimental cirrhosis by carbon tetrachloride.

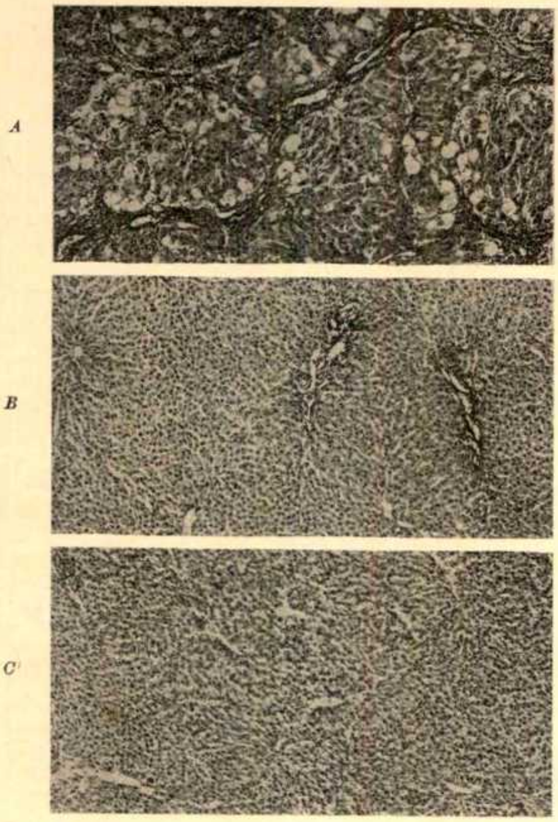

Fig. 1. *A*, Rat liver after 60 days of treatment with carbon tetrachloride; *B*, rat liver after 60 days of carbon tetrachloride and 53 days of AAN treatment; *C*, normal rat liver. Van Gieson staining. (× *c*. 31)

100 female rats weighing 150–200 gm. were used. Cirrhogenic treatment was given daily by inhalation, all the animals simultaneously breathing the same atmosphere with carbon tetrachloride vapour.

The cages containing the animals were put into a box where carbon tetrachloride was allowed to fall in drops on the plate of an electric oven until the vapour reached an approximate concentration of 50 gm. per m.^{3}. The dropping velocity was regulated so that this concentration of carbon tetrachloride vapours was attained after 7–8 min. Immediately after, the cages were removed from the box.

After the first week's treatment with carbon tetrachloride the rats were divided into two groups of equal number. One group was treated daily, in addition to carbon tetrachloride, with a subcutaneous injection of 20 mgm. aminoacetonitrile hydrosulphate dissolved in 0·2 ml. water and neutralized with sodium hydroxide. The other group, used as control, continued to receive daily treatment with carbon tetrachloride. After one month, at varied intervals, an equal number of animals from the two groups were killed and liver sections were histologically examined, having been stained with Van Gieson, Azan Mallory, hæmatoxylin and eosin. After two months the experiment was ended by killing all the surviving animals.

Both in the group treated with carbon tetrachloride and in that treated with carbon tetrachloride and AAN a high rate of mortality was observed, probably due to excessive amounts of the carbon tetrachloride used. Starting from the thirtieth day of treatment with carbon tetrachloride, livers were examined histologically, when possible, also from animals which were found dead.

Histological examination of the liver was carried out on a total of 25 animals treated with carbon tetrachloride and 24 animals treated with carbon tetrachloride and AAN. Between the thirtieth and fortieth days of carbon tetrachloride administration the newly formed connective tissue begins to be evident in the liver of animals treated with carbon tetrachloride alone, being detectable around the blood-vessels and in trabeculæ projecting among the lobules. From this moment the newly formed connective tissue is progressively increasing. After the sixtieth day's treatment, the cirrhosis picture is well marked. Big connective trabeculæ rich in fibroblasts and histiocytes surround the lobules, in which parenchymal cells show the typical severe alterations which follow carbon tetrachloride treatment (Fig. 1*A*). At this stage cirrhosis reveals itself macroscopically too, in that the liver acquires a clearly granular surface. On the contrary, in the liver of all the animals treated with carbon tetrachloride and AAN, including those which had received carbon tetrachloride for 60 days, the formation of collagen is extremely reduced: in comparison with the normal liver (Fig. 1*C*) it is slightly increased almost exclusively around the blood-vessels (Fig. 1*B*). Parenchymal cells are apparently normal.

The number of fibroblasts and histiocytes is also greatly reduced: this result does not agree with those of previous investigations[1-4] in which the action of lathyrogenic agents on the development of connective tissue was studied in different experimental conditions. It was then observed that lathyrogenic agents exert a delaying action on collagen formation, but do not inhibit the multiplication of fibroblasts and histiocytes. The present observations would suggest that the inhibitory effect of AAN on hepatic fibrosis due to carbon tetrachloride is not only due to the already known specific inhibition of collagen formation, but also to an inhibition of fibroblast multiplication.

Since the present observations do not exclude the possibility that AAN by some unknown interaction with carbon tetrachloride may prevent the latter substance from exerting its cirrhogenic activity, experiments are being made to ascertain whether it inhibits also fibroses of the same organ arising from other causes.

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ANATOMY

Continuous Regeneration of the Dentinal Nerve-Endings

IN recent years several investigators have reported that nerve fibres have been observed growing from the dental pulp into the so-called predentine[1-5]. These authors all dealt with human dentine. I published[6] last year an account of work showing the presence of unmyelinated nerve fibres in the predentine and the tubules of the dentine also of the rat. The occurrence of these fibres in rat dentine is very irregular, and seemingly certain spots are preferred. The distribution of the sensory nerves in the higher mammals is very similar, with the exception, of course, that the pulp innervation of the continuously growing incisor[7], and other continuously growing teeth[6] of rodents follows a quite different pattern. I have examined freshly extracted young human teeth also, demonstrating the nerve fibre in the dentine by the slightly modified method described by Romanes[8] using frozen sections. I found, in agreement with Weddel and Zander[9], that *p*H and length of time in the different fluids are of importance.

In some preparations I could clearly observe and confirm the presence of nerve fibres, mostly in the predentine, and also in some specimens the presence of a widely distributed nerve net in the predentine. The nerve fibre cannot be followed regularly in the calcified dentine by the methods of Romanes or Holmes; apparently this is possible only in very rare instances, as in the case of Powers[3]. I could follow some nerve fibres for a short way at the border of the calcified dentine, where they become indistinct. It seems to me that in the calcified dentine there are incorporated nerve fibres which are rarely distinguished with our present techniques. There is a possibility also that they become calcified and cease to function as receptors.

The dentine of higher mammals develops during the so-called functional period of the tooth also; the dentine becomes thicker and the pulp chamber narrower. The greater part of the dentine becomes calcified, although the inner—newly developed ground substance—is for a while uncalcified. This mechanism goes on until the formative power of the odontoblasts is exhausted. On the outer border of the uncalcified dentine, calcification goes on —spreading mostly in a spherical arrangement—and on its inner border new collagen ground substance is laid down.

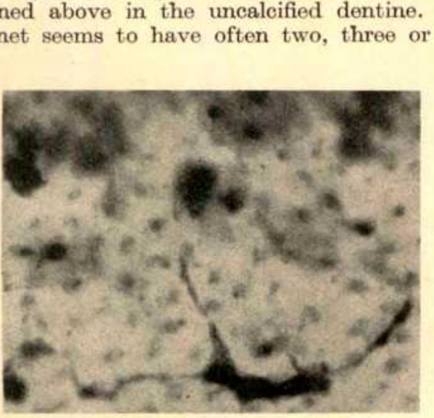

Fig. 1. Predentine, cross-section, showing a 'varix' with three nerve fibres originating from it. Left upper second premolar, girl, 14-years old. × 920

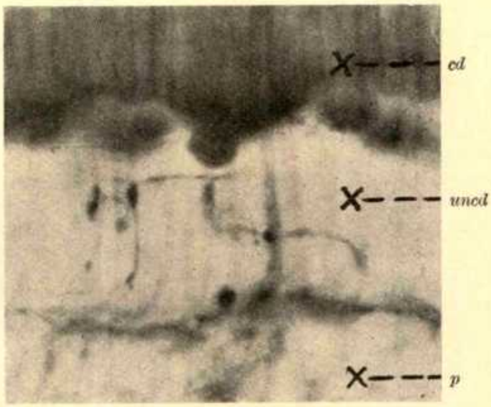

Fig. 2. Nerve fibres in the predentine running in three layers. From the same tooth as in Fig. 1. *cd*, Calcified dentine; *uncd*, uncalcified dentine; *p*, pulp; × 750

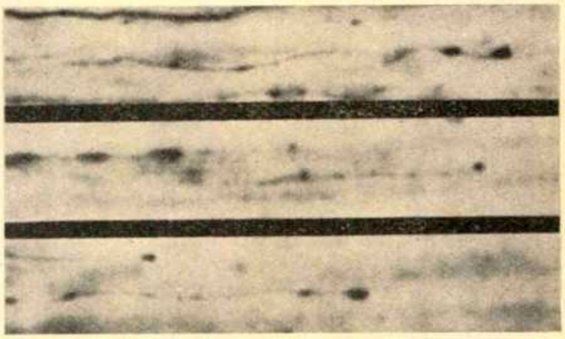

Fig. 3. Calcified dentine from a left lower second incisor of a boy 12 years old; longitudinal section. Three strips of dental tubuli with nerve fibres in it. × 1,200

I found that in the crown-pulp of human teeth the distribution of the unmyelinated nerve fibres in the uncalcified dentine or predentine shows a more or less regular pattern. I believe that some of these fibres come to a 'stop' on or near the inner border of the uncalcified dentine and develop there a 'varix' sometimes of considerable size (Fig. 1) and then, perhaps by active ingrowth, produce the nerve net mentioned above in the uncalcified dentine. This nerve net seems to have often two, three or more layers (Fig. 2); parts of the nerve net can also be found on the lowest layer, that is, on the inner border itself. Connexions between the different layers may be very numerous and of very different kinds. Some of the nerve fibres can be followed in the dentinal tubules, where they proceed with the odontoblastic process in the distal direction. Most of the fibrils are dotted or beaded at regular intervals in the uncalcified dentine as well as in the dentinal tubules (Fig. 3), and are, as in the case of the calcified dentine, only in this way discernible because the nerve fibres themselves become very thin.

All this—and the fact that 'varices' can be found at different levels or layers—suggests, in my opinion, the following conclusion. The peripheral part of the unmyelinated nerve fibre is, at least partly, in a state of continuous regeneration, that is, development. (Peripheral regeneration of the nerves is a common experience and is known also in newly formed tissues in the region of the teeth[10] in the case of injuries but not on a physiological basis.) It may be that the functioning recipient parts of the nerve fibre are the dotted ones, in the odontoblastic area, in the uncalcified dentine and in the dentinal tubules. With the centripetal calcifying of the dentine matrix, the nerve fibre of the predentine becomes incorporated in the calcified part and ceases to function; there develops eventually from the same fibril a branch at a lower level; perhaps repeatedly. If such a fibril is exhausted, then a new one grows in the uncalcified dentine, building a varix at the termination of the pulp. The varix becomes in this way itself involved in the developing ground substance. This explains why 'varices' can be found deep in the uncalcified dentine.

It seems highly likely that the mechanism briefly described here is not restricted to dentine, but is a regular pattern of the different subcutaneous free receptors, at least in the mucosa and in the skin.

A more detailed report of this work will be published elsewhere.

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BIOLOGY

Promotion of Cucumber Hypocotyl Growth by Two New Gibberellins

Wittwer and Bukovac[1] and Lockhart and Deal[2] have recently shown that gibberellin A_4, though less active than gibberellin A_1 or gibberellic acid in most bioassay systems[1,3,4], is far more active than A_1 or gibberellic acid in promoting the growth of cucumber stems. We have found that two recently discovered gibberellins, A_7 and A_9, both produced by *Gibberella fujikuroi*[5,6], also show specifically high activity in promotion of cucumber hypocotyl growth.

Cucumber seeds were germinated on moist filter paper at 25° C. in darkness. After 48 hr. the seed-coat was removed from germinated seeds and they were transplanted in dishes of 1 per cent water agar. The gibberellin was applied in 2-µl. drops of ethanol to the cotyledons. After 2 (Table 1) or 3 (Table 2) days growth at 25° C. in diffuse white light, the lengths of the hypocotyls were measured. The results of experiments with two varieties of cucumber, in which gibberellins A_4 and A_7 and gibberellic acid were compared, are presented in Table 1. It will be seen that both A_4 and A_7 are considerably more effective than gibberellic acid in promoting hypocotyl growth. We have found that gibberellin A_7 is of the same order of activity as gibberellic acid in promotion of lettuce hypocotyl growth, but less active than gibberellic acid in promotion of pea stem extension.

Gibberellin A_9 is much less active than gibberellic acid in promotion of pea stem or lettuce hypocotyl growth; with both plants a dose of 10 µgm. A_9 is required to give an effect comparable to that of 0·1 µgm. gibberellic acid. Nevertheless, A_9 is much more active than gibberellic acid in promotion of cucumber hypocotyl growth (Table 2). In other experiments we have confirmed the observation of Wittwer and Bukovac[1] that gibberellin A_1 is of the same order of activity as gibberellic acid on cucumbers.

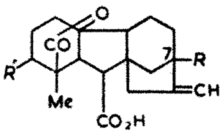
Table 1. Hypocotyl Length (mm.) of Cucumber Seedlings (Means of 20 Replicates ± *S.E.*)

Dose (µgm./plant)	Long Green ridge cucumber Gibberellic acid	Long Green ridge cucumber A_4	Long Green ridge cucumber A_7	Perfection ridge cucumber Gibberellic acid	Perfection ridge cucumber A_4	Perfection ridge cucumber A_7
0·2	15·7 ± 0·6	32·0 ± 1·4	33·3 ± 1·5	15·4 ± 0·8	28·7 ± 1·2	30·4 ± 1·5
1·0	17·5 ± 1·0	32·5 ± 1·5	32·3 ± 1·7	19·5 ± 1·4	30·1 ± 1·3	35·0 ± 1·5
5·0	21·2 ± 1·1	34·7 ± 1·9	29·9 ± 1·3	18·2 ± 1·5	28·5 ± 1·4	34·5 ± 1·6
Untreated controls	16·3 ± 1·0			12·4 ± 0·7		

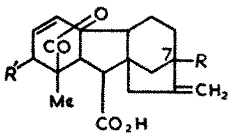
Table 2. Hypocotyl Length (mm.) of Cucumber Seedlings (Means of 30 Replicates ± *S.E.*)

Dose (µgm./plant)	Long Green ridge cucumber Gibberellic acid	Long Green ridge cucumber A_9	Perfection ridge cucumber Gibberellic acid	Perfection ridge cucumber A_9
0·2	27·2±0·9	41·3±1·1	33·4±0·9	42·1±1·0
1·0	30·5±0·8	50·9±1·2	37·2±1·0	51·1±1·3
5·0	40·8±1·0	55·8±1·4	41·0±1·6	53·9±1·5
Untreated controls	25·3±0·7		26·0±0·8	

It is perhaps significant that gibberellins A_4 (I; $R = H, R' = OH$), A_7 (II; $R = H, R' = OH$) and A_9 (I; $R = R' = H$) have one structural feature in common that distinguishes them from A_1 (I; $R = R' = OH$) and gibberellic acid (II; $R = R' = OH$), namely, the absence of a hydroxyl group at position 7.



(I)



(II)

We hope shortly to compare these gibberellins in other bioassay systems where A_4 has been shown to have specific activity[7]. We are indebted to Dr. B. E. Cross for samples of gibberellins A_4, A_7 and A_9.

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Effect of High-Temperature Pre-Treatment on the Germination of Oil Palm Seed

It has been known for more than forty years that a high temperature is essential for the germination of oil palm seed[1], and on a commercial scale seed is germinated in sand beds exposed to the Sun, in fermentation pits or in artificially heated germinators.

The optimum temperature for the germination of kernels was shown by Hussey[2] to lie between 38 and 40° C., and a temperature of 39·5° C. has been successfully adopted for germinating seed in a specially designed germinator[4].

It has also been shown[3] that an 80-day period of high temperature is sufficient for the germination of fresh seed; periods longer than this have no significant effect on the percentage germination obtained. When the seed is kept at ambient temperature (about 27° C.) after heat treatment, the rate of germination is characteristically rapid, 80 per cent germination being obtained in about 8 days, and complete germination within 15 days. If, however, the moisture content of the seed during the heat treatment is below the optimum for germination, the rate of germination at ambient temperature is lower and complete germination may take up to 6 weeks.

In a recent experiment, four 600-ml. jars covered with 0·002 in. polythene sheet and containing 50 *dura* (thick-shelled) seed were kept for 80 days at 39·5° C. at each of the four moisture contents shown in Table 1. The first of these was achieved by over-watering and maintaining a ½-in. layer of water at the bottom of the jar. In treatments 2 and 3 the seeds were examined three times a week and watered when necessary, the moisture content being estimated visually, while in treatment 4 the seeds were dried at the start of the experiment and received no water during the heat treatment. The moisture content of the seed in treatment 2 was the optimum for germination. After 80 days all jars were brought to ambient temperature. In two jars from each treatment the seeds were soaked for 5 days, then dried slightly to the optimum moisture content, and in the remaining two the seeds were left unchanged. The germination percentages obtained, together with the moisture contents determined from samples taken after the heat treatment, are shown in Table 1.

Table 1. GERMINATION OBTAINED FROM FOUR BATCHES OF SEED GIVEN 80 DAYS HEAT TREATMENT AT 39·5° C. AT A RANGE OF CONSTANT MOISTURE CONTENTS. HALF THE SEED IN EACH BATCH WAS THEN BROUGHT TO OPTIMUM MOISTURE CONTENT FOR GERMINATION AND HALF WAS LEFT UNCHANGED

Seed batch	Moisture content (per cent) Nominal	Moisture content (per cent) Actual	Germination (per cent) at Original moisture content	Germination (per cent) at Optimum moisture content	Days to 50 per cent of final germination at optimum moisture content after 80 days
1	24	24·2	12	71	28·5
2	21 (optimum)	21·3	95	92	6·5
3	19	18·6	0	97	17·5
4	14	14·2	0	97	25·0

It will be seen that excellent germination may be obtained from heat-treated air-dry seed afterwards cooled at optimum moisture content, although following cooling there is a time lag before germination starts which accounts for the differences in rates of germination shown for batches 2, 3 and 4 in Table 1. In large-scale germination of seed, thrice-weekly examination of seed for watering during the heat treatment period is rendered unnecessary, and, more important, the risk of over-watering, which is more dangerous to the final germination than under-watering, is eliminated.

This high-temperature requirement of seed, a pretreatment which corresponds to the well-known low-temperature stratification of seed of temperate plants, is remarkable in that it can be satisfied at a low seed moisture content.

In a region such as Southern Nigeria with a severe 3–4 month dry season, a high-temperature requirement has an obvious survival value, ensuring germination when water becomes available for growth.

This work is continuing and will be reported in fuller detail in the Institute's Journal.

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Effect of Temperature Fluctuations on Flowering in *Salvia splendens*

IN the majority of experiments that have been carried out on the interaction of day and night temperature on plant growth, a certain day temperature is selected and this is compared with varying night temperatures or vice versa. From the results of such experiments[1,2] the explanation offered for any particular combination of temperature treatments proving more favourable for the development of the plant usually rests on the hypothesis that one or more of the processes susceptible to temperature change, for example, photosynthesis, respiration or translocation, is furthered or hindered during night or day. Therefore, depending on the suitability of the temperature combination with the period of illumination, the growth of the plant is similarly furthered or hindered.

In experiments carried out in the phytotron of the University of Liège[3] using a long-day flowering variety of *Salvia splendens* Sello it was found possible to produce the same response in the growth and flowering of the plant through using combinations of day and night temperatures that were the opposite of one another.

Salvias were grown at 20 and 27° C. keeping the day and night temperature constant in both cases. Plants were also grown in long days with the temperature kept at 20° C. by day and 27° C. by night, and vice versa. The changes in temperature were controlled by thermostatic clocks, thus avoiding the necessity of moving the plants abruptly from one temperature to another. In all the temperature treatments the relative humidity was kept at 80 per cent.

Table 1 compares the growth and flowering of the plants grown in 16-hr. days under the different temperature conditions.

It is seen that there is little difference in the time taken for the plants to flower or in the height of the

Table 1. COMPARISON OF STATE OF DEVELOPMENT OF SALVIA PLANTS GROWN IN 16-HR. DAYS WHEN 50 PER CENT OF THE PLANTS IN EACH TREATMENT (150 PLANTS PER TREATMENT) HAVE JUST PRODUCED THEIR FIRST FLOWERING BUD

	20° C. day and night	27° C. day and night	20° C. day and 27° C. night	27° C. day and 20° C. night
Age in days	36	38	49	53
Height (cm.)	4·6	4·6	6·3	8·6
Vegetative nodes formed	6	8	8	9

plants at flowering when the temperature is kept at 20 or 27° C. constantly day and night. However, when the temperature is allowed to vary either 20° C. by day with 27° C. by night or vice versa there is a retardation in the flowering of the plants. With both these temperature treatments the response is the same, the plants being both older and taller at flowering.

Explanations based on such hypotheses as used by Went *et al.*[1,3] are here of no avail, as opposite conditions produce in each case the same result. It can only be concluded that the alternation of temperature produces its effect without being correlated with either the period of illumination or darkness.

I am indebted to Prof. R. Bouillenne for facilities in the phytotron, to Dr. C. Sironval for advice and to the Carnegie Trust for the Universities of Scotland for a scholarship.

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Biochemical Classification of Yeasts

Roberts and Thorne[1] disagree with my proposals[2] for improving the classification of yeasts, but themselves suggest no way of overcoming the difficulties (which they acknowledge exist) in using the present system[3]. Instead, they emphasize the desirability of constructing a phylogenetic classification; though this is obviously impracticable.

I advocated developing a classification of yeasts based mainly, not, as they claim, "exclusively", on the biochemistry of yeasts. Roberts and Thorne also imply that I hold the view that because morphological differences reflect chemical ones, morphology is neither interesting nor important. This argument is a *non sequitur* and I did not put it forward.

It is strange that Roberts and Thorne object to the idea that the most important thing about yeasts is their biochemical activity. Chemical processes determine the role of yeasts in brewing, wine-making, baking, nutrition, spoiling foodstuffs and as pathogens. Thus it is not surprising that of thirteen papers on yeast genetics published by Winge and Roberts[4] between 1948 and 1958, at least nine were concerned with biochemical characteristics.

In the present lack of knowledge about the ancestral history of yeasts, it is self-deception to say that any classification of them is phylogenetic. The idea of chronology is implicit in any phylogenetic classification; and this has little meaning except in terms of the age of ancestral forms[5]. Kudriavzev's[6] and Zsolt's[7] theories of evolutionary relationships are based on insufficient evidence; and Wickerham's speculations[8] on the phylogeny of the *Hansenulae* involve false assumptions[9]. Kudriavzev held that it was necessary for his classification to be phylogenetic. Because of the difficulty in developing a phylogenetic theory for the asexual forms (p. 80), he did not publish any data on the species of non-sporing yeasts, and this detracts enormously from the value of his book[6].

The Dutch classification of yeasts[3] is by far the most complete and therefore the most useful. As I have pointed out before[2,9], it has, however, three major weaknesses. (1) Sporulation tests are given supreme importance. These involve a bewildering array of media (Phaff and Mrak[11], list 18) and extensive searching for spores. Moreover, since a negative result is equivocal, these tests are very unsatisfactory. (2) The morphological criteria used, are ill-defined and difficult to specify precisely. (3) The biochemical tests are excessively crude and hence uninformative.

Roberts and Thorne do not say that these criticisms are invalid. Assuming their validity, it seems reasonable to develop techniques for classifying yeasts in which: (*a*) there are no sporulation tests; (*b*) the morphological criteria are given greater precision; (*c*) biochemical tests are designed to give more information.

I do not suggest that studies of morphology or of sporulation should stop; but I do suggest that in classification biochemical criteria should be given more importance than at present. No embargo on evolutionary speculation has been proposed; but there is no reason why such conjecture must be applied to yeast taxonomy. Perhaps it is worth noting van Niel's striking *volte-face*: although in 1936 he held that bacterial classification had to be phylogenetic[12], ten years later he wrote: "Bacterial taxonomy is far more similar to Linnaeus's original system of the plants. . . . Both kinds of system are useful if they permit the rapid assignment of a specimen to a restricted group, but neither can lay claim to representing phylogenetic relationships", and in another passage: "It is not hereby implied that such systems are inferior to the 'natural' systems of the botanist and zoologist, but merely that they serve a different purpose, and are arbitrary"[13].

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Effects of Light and Optical Sensitization by Acridine-Orange on Living Bull Spermatozoa

Continuation of investigations on the effects of vital fluorochroming of bull spermatozoa with acridine-orange[1] has now confirmed the view that the observed adverse effects may be attributed to photodynamic action exerted by the fluorochrome.

Bull semen was diluted with optically clear egg-yolk – citrate buffer, every sample being split into

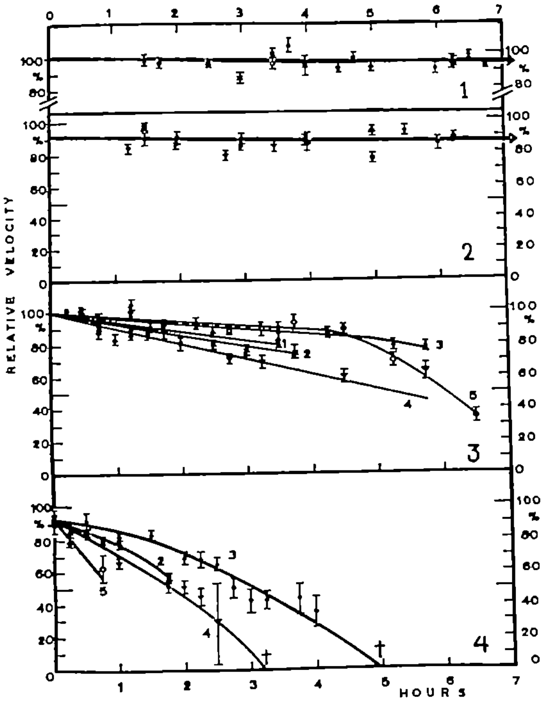

Fig. 1. Relative mean velocities of bull spermatozoa as a function of time, with and without acridine-orange.

Curve 1. Controls without acridine-orange, kept in darkness, initial mean velocities have been taken as 100 per cent; the absolute values were 107 ± 11 μ/sec. (*S.E.* ± 5).

Curve 2. Samples with acridine-orange, kept in darkness. Initial velocity 92 per cent relative to the initial mean velocity of the controls, as a result of the adverse effect of photosensitization during the measuring procedure.

The regression lines of (1) and (2) were calculated from data extending to 30 hr., only the first 7 hr. being shown here. The spread of the curves obtained in the individual experiments being very small, only the regression lines for pooled data are shown. Curve 3. Controls without acridine-orange, kept under illumination with white light. Initial mean velocity taken as 100 per cent. Curve 4. Samples with acridine-orange, kept under illumination. At † no actively swimming specimens could be observed visually under the microscope

four portions, to two of which acridine-orange in a final concentration of 1 : 50,000 was added. The darkness samples were kept in a Dewar vessel with ice, the tubes being isolated from direct contact with the ice by an air mantle. The samples used for irradiation (also isolated by an air mantle) were placed in a glass beaker with ice, separated from the light source (a 220-V., 200-W. incandescent lamp) by a cooling cylinder filled with water and ice. At regular intervals drops were taken from the test-tubes and brought into the photo-electric equipment[1-4].

With the samples kept in darkness, there was no statistical difference between the semen with acridine-orange and the control with respect to the rate of decrease of velocity, and the rate of decrease of the number of spermatozoa moving normally; however, the curves were shifted to a lower level. The amount of this constant difference per ejaculate could be accounted for completely by the effect of optical sensitization by acridine-orange during the period of measurement. The mean rates of decrease of velocity with time were 0·55 ± 0·18 (*S.E.*) μ/sec./hr. for the controls and 0·56 ± 0·17 μ/sec./hr. for the samples with acridine-orange.

In the illuminated samples the mean velocity of spermatozoa with normal movement decreased rapidly in comparison to the samples kept in darkness, the effect of addition of acridine-orange being most striking (Fig. 1). All curves bend towards the abscissa. From an analysis of much more extensive material on the rates of decrease of velocity under different conditions, it may be expected that all the curves will bend down to the abscissa if the period of taking measurements is sufficiently extended. The curves can be described by the equation:

$$\bar{v}_t = \bar{v}_0 - \alpha t.\exp(kt) \qquad (1)$$

where $\bar{v}_t$ is the mean velocity at an interval of time t after the beginning of the experiment when the mean velocity is $\bar{v}_0$; α and k are constants determining slopes and bending points. If the numerical value of kt is small, (1) reduces to the straight-line equation:

$$\bar{v}_t = \bar{v}_0 - \alpha t \qquad (2)$$

obtained in experiments of short duration relative to the rate of decrease of velocity.

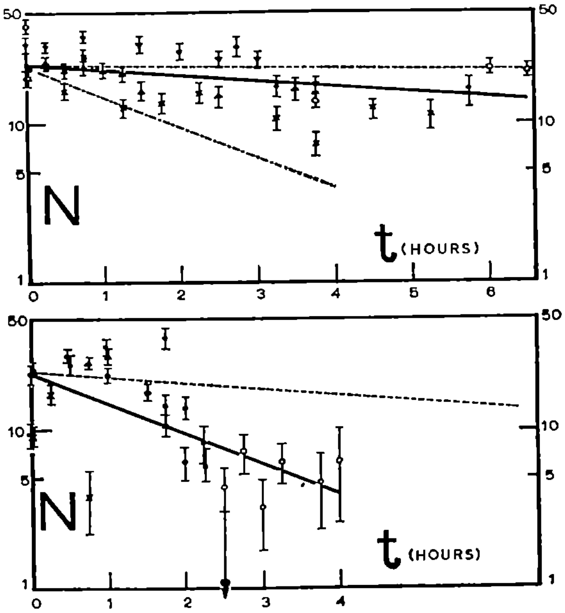

Fig. 2. Semi-logarithmic plots of numbers *N* per volume (in arbitrary units) of bull spermatozoa moving normally, as a function of time, under illumination with white light.

Upper graph: Controls without acridine-orange. The upper dotted line indicates the curve obtained for the samples kept in the dark, while the lower dotted line indicates the curve for samples with acridine-orange added kept under illumination. These dotted curves may facilitate comparison.

Lower graph: Samples with acridine-orange added. The dotted line indicates the curve of the illuminated control

The number *N* per unit volume of spermatozoa moving normally has been found to decrease exponentially with time during the limited periods actually investigated[3-6], according to:

$$N_t = N_0.\exp(-kt) \qquad (3)$$

yielding a straight-line relationship on a semi-logarithmic plot (Fig. 2). Whether this relationship would hold over an extended range is doubtful, since according to (3) the number of moving specimens would only become zero at infinite time, which is obviously impossible, the last specimen in reality becoming immotile at a very finite time indeed. With this restriction, the values of k and the half-life periods $t_{1/2}$ obtained for the four different conditions were:

Samples without fluorochrome, in the dark: $k = 0{\cdot}018$ hr.$^{-1}$, $t_{1/2} = 38$ hr.

Samples without fluorochrome, illuminated: $k = 0{\cdot}078$ hr.$^{-1}$ $t_{1/2} = 8{\cdot}8$ hr.

Samples with acridine-orange, in the dark: $k = 0{\cdot}017$ hr.$^{-1}$, $t_{1/2} = 40$ hr.

Samples with acridine-orange, illuminated: $k = 0{\cdot}43$ hr.$^{-1}$, $t_{1/2} = 1{\cdot}6$ hr.

There is no significant difference in slope between the curves obtained for the samples with and without acridine-orange kept in darkness ($P > 0{\cdot}90$). The slopes of both curves differ significantly from a horizontal line ($P < 0{\cdot}001$).

The slope of the curve of the samples without acridine-orange, kept in darkness, differs significantly from that of the illuminated portions of the same samples ($0{\cdot}02 < P < 0{\cdot}05$), illumination increasing the rate of reduction of the number of spermatozoa.

The slope of the curve of the illuminated samples with acridine-orange is significantly steeper than that of the curves obtained for the samples kept in darkness ($P < 0{\cdot}001$), and also than that of the curve obtained for the samples with acridine-orange kept in the dark.

These results confirm that the number of normally moving spermatozoa in diluted semen without the addition of fluorochrome decreases much faster under the influence of illumination than in darkness, in agreement with previous findings by Norman and Goldberg[7], whose results, however, do not allow further analysis of the relationship since evaluations were performed at two time intervals only. The ratio of the slope constants in these two conditions is obviously dependent on the actual level of illumination employed. This is not the case with a comparison between the constants for the slopes obtained for the same illuminated samples with and without acridine-orange, since both are then exposed to the same light intensity. Whereas in darkness acridine-orange (1 : 50,000) did not have any demonstrable effect on the mean velocity and the number of normally moving sperms during a period of storage in a Dewar vessel with ice up to 30 hr., a big and deleterious effect in both respects occurs in the samples kept under illumination; this effect is enhanced considerably by photosensitization by acridine-orange.

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Size of Particles ingested by *Simulium* Larvæ

THE diet of the aquatic larval stages of the blackfly, *Simulium*, some adults of which transmit onchocerciasis to man and animals, has been described; but little information is available on the size of the particles ingested.

The size of particulate matter taken in by *Simulium* larvæ has been measured for four temperate species of *Simulium*.

S. ornatum was obtained from a small highly calcareous stream at Hawarden, Flintshire. This stream is heavily shaded by trees and slightly polluted by farm drainage.

S. ornatum, *S. reptans* and *S. variegatum* were collected from the River Ceirog at Chirk, Denbighshire. This river, a tributary of the Dee, is shaded by trees, is moderately calcareous, and is slightly polluted from cattle pasture.

S. tuberosum was obtained from the Hirnant, a soft-water hill stream at Bala, Merionethshire.

The contents of the midgut were separated from the gut tissue on a slide, and spread by gentle pressure on a coverslip. The longest and shortest axes of 45 randomly selected particles were measured in 5 last instar larvæ in each group, and in 5 earlier instar larvæ which were not identified, taken from the Ceirog.

The frequency distribution of particle sizes in each group was log normal, and the sizes in the individuals of the different species from the different rivers were similar.

(*S. ornatum*, Hawarden, 11·3 ± 1·8μ, 6·6 ± 1·7μ; *S. ornatum*, Ceirog, 13·2 ± 2·1μ, 7·3 ± 1·8μ; *S. reptans*, 15·1 ± 2·0μ; 8·0 ± 1·8μ; *S. variegatum*, 14·8 ± 2·1μ, 7·8 ± 1·9μ; *S. tuberosum*, 12·0 ± 1·9μ, 7·7 ± 1·9μ; unidentified early instars 14·0 ± 1·7μ, 7·3 ± 1·7μ.)

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BACTERIOLOGY

Chelating Agents for Selective Growth of Bacteria

THE use of *p*H buffers for stabilizing the *p*H of growth media has become common bacteriological practice. There do not appear to be any reports, however, of the similar use of chelating agents for controlling the concentration of metal ions as 'metal buffers'[1,2]. With metal ions there is a more complex situation, of course, since there are few chelating agents selective for one particular metal. An observed effect on bacterial growth can, therefore, be due to simultaneous sequestration of several metal ions and this must be allowed for in designing experiments.

It has been found that, by suitable control of the concentration of magnesium ions, liquid and solid media can be devised for selection of coagulase-positive staphylococci in presence of coagulase-negative strains and of many commensal organisms. By calculation from the controlled *p*H and from the known stability constants of the chelating agents[3] employed, it is found that the concentration-range over which an effect can be observed in the growth-rate of coagulase-positive staphylococci lies roughly between $M/1{,}000{,}000$ and $M/25{,}000$ free magnesium ions (or if expressed more conveniently in terms of the negative logarithm, from *p*Mg 6·0 to 4·4). This accords with the value of $<M/250{,}000$ to $M/25{,}000$

found by Shooter and Wyatt[4] by returning magnesium and calcium ions to media previously exhausted by treatment with '*IRC*-50' ion-exchange resin.

A representative liquid medium which will distinguish coagulase-positive from coagulase-negative strains of staphylococci (picked from colonies on blood agar plates) after 6–24 hr. incubation at 37° C. is as follows: 'Casamino' acids (Difco), 0·2 per cent; yeast extract ('Oxoid'), 0·05 per cent; Na_2HPO_4, 0·0144 *M*; KH_2PO_4, 0·0056 *M*; Na_3H salt of ethylenediamine tetraacetic acid (Na_3HEDTA), 0·0012 *M*; Na_2 [MgEDTA], 0·006 *M*; Na_2 [CaEDTA], 0·007 *M*; Na_2 [MnEDTA], 0·001 *M* (added phosphate 0·02 *M*, *p*H 7·2, calculated *p*Mg 4·8).

A representative agar medium which can be used for selection of coagulase-positive colonies in primary cultures from a slit sampler, or from sweep inocula from swabs or like sources, is as follows: Nutrient agar (Difco), 3·4 per cent; Na_2HPO_4, 0·0144 *M*; KH_2PO_4, 0·0056 *M*; Na_3HEDTA 0·00325 *M*; Na_2 [MgEDTA], 0·01 *M*; Na_2 [CaEDTA], 0·008 *M*; Na_2 [MnEDTA], 0·001 *M* (added phosphate 0·02 *M*, *p*H 7·2; calculated *p*Mg 4·6).

These recipes make allowance for the free metal ions originally present in the nutrient media used, and might require modification if different nutrients with different contents of metal ions were employed.

An early application of the agar medium has been in the detection of coagulase-positive staphylococci in pillows and mattresses (Knights, H. T., unpublished work), cultures from which are commonly overgrown by other organisms. Work on further applications of chelating agents is continuing.

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Plasma Membranes and Mitochondrial Equivalents as Functionally Co-ordinated Structures

A controversy has existed since 1951 concerning the sites of oxidative-reductive reactions in the bacterial cell. Several investigators have demonstrated biochemically the presence of oxidative-reductive systems in particulates derived from the bacterial cytoplasmic membrane and probably also from contiguous granules. Weibull[1], Mitchell and Moyle[2], Storck and Wachsman[3], Weibull and Bergstrom[4], Keeler, Carr and Varner[5], Cota-Robles *et al.*[6] and Weibull *et al.*[7] have presented evidence that the cytoplasmic membrane, its fragments and associated granules contain most of the cytochromes and enzymes responsible for succinate, reduced diphosphopyridine nucleotide, DL-lactate, and α-ketoglutarate oxidation and about half the L-malate oxidative activity.

Evidence of oxidative-reductive events localized in cytoplasmic granules has been advanced by Bielig, Kausche and Haardick[8], Kellenberger and Huber[9], Georgi *et al.*[10] and Mudd *et al.*[11]. A considerable literature has grown up in support of each view. The purpose of this communication is to indicate that the reality of the situation lies rather in integration than in continuing opposition of these views concerning the sites of bacterial oxidation-reduction.

In the present work strains of *Bacillus megaterium* were examined as bacterial cells, as protoplasts and as ghosts, with the light and the electron microscopes. Cell walls, septa, cytoplasmic membranes and contiguous granules were clearly visualized. Reduction of various tetrazolium salts, serving as oxidation-reduction indicators, occurred in the protoplasts very rapidly, often becoming microscopically visible immediately after addition of the indicator.

Tetrazolium salts were sometimes added to the intact bacilli in sucrose solution without lysozyme. When granules of the reduction product had become visible microscopically, lysozyme was added, thereby converting the granule-containing bacilli into granule-containing protoplasts, showing that the tetrazolium granules of the bacilli and the protoplasts were one and the same and that the granules were not associated with the cell wall. When protoplasts containing formazan granules were centrifuged and distilled water was added in place of sucrose to cause rupture of the protoplasts, the irregular, shattered membranes could be seen in wet mounts to be associated with the stained granules.

Reduction of potassium tellurite proceeded slowly in both bacilli and protoplasts and usually required several hours to produce granules; the resulting sites appeared to be similar to those produced by reduced tetrazolium salts. The protoplast membrane took on a greyish shade after long exposure to tellurite. The granules, which appear as homogeneously dark particles in the light microscope, were seen in the electron micrographs to be localized deposits of tellurium crystals.

For observation in the electron microscope cells were grown in a medium containing potassium tellurite and studied either as intact cells or as ghosts or after ultra-thin sectioning. Such preparations were fixed in buffered formaldehyde to avoid re-oxidation of the reduced tellurium by the usual fixatives. In such preparations, crystals of the reduction products were seen throughout the cells. In sections clusters of crystals were seen at sites near the periphery of the cytoplasm, and as individual crystals or amorphous grains in the periphery of the ghosts. The persistence of reduction products in the ghosts strongly suggested that the sites of reduction were in or in close association with the plasma membrane. These results are compatible with the interpretation that oxidation-reduction reactions occur both in the plasma membrane and in associated cytoplasmic organelles.

In offering a definition of bacterial mitochondria in 1956, the senior author concluded with the following sentence[11]: "There is at present no positive evidence of the existence of double membranes or *cristae mitochondriales* in bacterial mitochondria". In the intervening years electron micrographs of lamellated cytoplasmic organelles have been published by a number of authors[12-23].

The nature of the cytological association between cytoplasmic membranes and contiguous cytoplasmic organelles has most recently been defined with even greater precision in at least one genus of Eubacteria and in Actinomycetales. In electron pictures of ultra-thin sections of *Mycobacterium tuberculosis*[14,15], of *Bacillus subtilis*[16] and of *Streptomyces coelicolor*[27] the lamellæ of the cytoplasmic membranes are seen to be continuous with the outer lamellæ of cytoplasmic organelles. In most recent work by Giesbrecht[28] on *Bacillus megaterium* 'organized' mitochondria are demonstrated the interiors of which are filled with closely packed *tubuli mitochondriales*. These mitochondria closely resemble the mitochondria of protozoa. It is greatly to be hoped that the biochemical relationships of these structures may soon be defined with equal elegance.

Preliminary accounts of this work have already appeared[29]. The work was aided by a grant from the United States Atomic Energy Commission, AEC Contract No. *AT*(30–1)–1342, and by Contract No. *E*–2690 with the Public Health Service, National Institutes of Health.

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GENETICS

Lethal Mutations in Brazilian Human Populations

A SURVEY has been made in some socio-economically similar rural populations in three neighbouring townships in the southern region of the central State of Minas Gerais, Brazil. About 1,100 families of unskilled workers and a few small farmers were studied. These families have been classified according to the ethnic background of the spouses and grouped into six categories: White × White ($W \times W$), White × Mulatoes ($W \times M$), White × Negroes ($W \times N$), Mulatoes × Mulatoes ($M \times M$), Mulatoes × Negroes ($M \times N$), and Negroes × Negroes ($N \times N$). The Whites are almost exclusively of Portuguese ancestry. Within each class, families were grouped according to the coefficient of inbreeding f of their offspring; these values ranged as follows: 1/8, 3/32, 5/64, 9/128, 1/16, 3/64, 17/512, 1/32, 3/128, 1/64, and 0 (assumed to be non-related). A number of families where the values of f were known to be lower than 1/64, but the precise values could not be ascertained with certainty have been discarded.

In the summary presented in this communication (details will be given elsewhere), only mortality within the group with $f = 1/16$, within the total sample of consanguineous marriages, and within the outbred control will be compared (Table 1). Mortality has been divided into three categories: (1) abortions plus miscarriages (from about the second up to and including the sixth month of pregnancy); (2) stillbirths; (3) mortality from birth up to and excluding the mean age of marriage (nineteen years for women and twenty-four for men). The frequencies of abortions plus miscarriages were calculated on the basis of pregnancies (twin pregnancies being taken as one); those of stillbirths, on the basis of children born (twin births were taken as two); and mortality after birth, on the basis of liveborn children. Mortality is not different among the five control samples ($\chi^2 = 7{\cdot}62$, for abortions plus miscarriages; $\chi^2 = 0{\cdot}52$, for stillbirths; $\chi^2 = 1{\cdot}74$, for post-birth mortality; $\chi^2 = 2{\cdot}80$, for the total). Among the five consanguineous samples, one value of χ^2 (3·86; stillbirths) is not significant; two values (9·20 and 9·22; abortions plus miscarriages and post-birth mortality, respectively) are rather close to the 0·05 level of significance; and one value (13·88; for the total) is highly significant ($P < 0{\cdot}01$).

Mortality is generally higher in the inbred groups than in the controls, but the differences are significant only in the $M \times N$ and $N \times N$ sub-samples: abortions plus miscarriages, $M \times N$, $\chi^2 = 7{\cdot}97^{**}$; mortality after birth, $M \times N$, $\chi^2 = 11{\cdot}70^{**}$; abortions plus miscarriages, $N \times N$, $\chi^2 = 11{\cdot}54^{**}$: stillbirths, $N \times N$, $\chi^2 = 4{\cdot}12^{*}$; total, $M \times N$, $\chi^2 = 12{\cdot}27^{***}$; total, $N \times N$, $\chi^2 = 5{\cdot}84^{*}$. The higher inbreeding effect in these two classes is not due to higher mean coefficients of inbreeding (Table 1).

Within each of the five groups of marriages, the inbreeding coefficients have been pooled, when possible, to form five classes as follows: 0; 1/64; 3/128 and 1/32; 17/512 up to and including 1/16; 9/128 up to and including 1/8. In situations where some of these classes were not present or the number of observations were too small (less than 25), coefficients were pooled in a different way according to the

Table 1. FREQUENCIES (PER CENT) OF MORTALITY IN THE CONSANGUINEOUS AND CONTROL SAMPLES

Couples		$W \times W$	$W \times M$ plus $W \times N$*	$M \times M$	$M \times N$	$N \times N$	Total
No. of pregnancies	Control Inbred† $f = 1/16$	2,375 621 280	1,187 187 58	988 157 53	357 68 37	595 69 32	5,452 1,052 460
F_s		0·009	0·005	0·006	0·007	0·005	0·007
F_c		0·045	0·046	0·046	0·045	0·044	0·045
Abortions plus miscarriages	Control Inbred† $f = 1/16$	10·40 10·47 8·57	11·52 8·03 10·34	9·41 12·74 9·43	7·28 16·18 24·32	8·74 20·29 18·75	10·07 11·50 10·65
Stillbirths	Control Inbred† $f = 1/16$	3·85 4·27 6·59	4·24 7·09 11·54	3·97 4·35 2·08	4·48 5·26 0·00	3·83 9·09 7·69	3·99 5·01 6·28
Mortality after birth	Control Inbred† $f = 1/16$	19·71 18·40 19·09	18·74 22·08 23·91	19·08 19·70 18·75	17·50 35·19 44·83	20·68 24·00 25·00	19·36 20·40 21·91
Total‡	Control Inbred† $f = 1/16$	31·12 30·27 31·07	31·31 33·58 39·66	29·86 33·12 28·30	27·17 48·53 56·76	30·59 44·93 43·75	30·61 33·27 34·78

* The two classes have been pooled because of the small number of pregnancies in the $W \times N$ consanguineous sample.

† Total consanguineous sample, including $f = 1/16$.

‡ Calculated on the basis of pregnancies.

F_s, Mean coefficient of inbreeding of the whole sample.

F_c, Mean coefficient of inbreeding of the consanguineous fraction. In both cases, the unit has been the pregnancy.

possibilities given by their distribution. Significant positive correlation between these pooled coefficients of inbreeding and inbreeding effect was found in ($W \times M$) plus ($W \times N$), stillbirths, $r = 0·95$* (d.f. = 2), and post-natal mortality, $r = 0·90$** (d.f. = 4); in $M \times N$, abortions, $r = 0·98$** (d.f. = 3), and post-natal mortality, $r = 0·95$** (d.f. = 3); and in $N \times N$, abortions, $r = 0·98$** (d.f. = 3).

Mean numbers of lethal equivalents carried by each person have been calculated according to the formula by Freire-Maia and Freire-Maia[1]. The estimates (Table 2) are suggestive of differences in the number of lethal equivalents in the two ethnic groups (Caucasians and Negroes) involved.

Negative values in Table 2 refer to situations where the frequencies of abortions (plus miscarriages) and mortality after birth were larger in the control group than in the consanguineous one. The negative total estimate for $W \times W$ ($-$ 0·37) turns out to be 0·06 when one uses only the data from 1/16 up to and including 1/8, and to 0·10 with data regarding only $f = 1/16$. Morton, Crow and Muller's approach[2] leads to $-0·18$ and Schull's variant[3] leads to 0·16 (Freire-Maia[4]).

Arbitrary values 1, 2, 3 and 4 were respectively ascribed to Whites, Light Mulatoes, Dark Mulatoes and Negroes. From these basic values, the five classes of marriages (in the order they are present in Tables 1 and 2) received the following values: 2·00, 3·35, 4·80, 6·48, and 8·00. The mean numbers of lethal equivalents estimated from the five groups of couples (that is, $-$ 0·37, 1·20, 1·89, 12·15 and 9·12) are positively correlated to the above values ($r = 0·87$*).

Table 2. MEAN NUMBERS OF LETHAL EQUIVALENTS PER PERSON

Couples	$W \times W$	$W \times M$ plus $W \times N$	$M \times M$	$M \times N$	$N \times N$	Total
Abortions	0·08	$-1·53$	1·46	3·95	5·24	0·68
Stillbirths	0·18	1·26	0·16	0·36	2·39	0·45
Mortality after birth	$-0·58$	1·47	0·27	7·84	1·49	0·47
Total	$-0·37$	1·20	1·89	12·15	9·12	1·55

The method developed by Morton, Crow and Muller[2] has been applied to our $W \times W$ and $N \times N$ data, and the results, rather close to ours, revealed a significant difference at the 0·05 level between the total estimates of B for the two ethnic groups. According to a method developed by Crow[5], it was concluded that, at least among Negroes, heterotic genes seem not to contribute substantially to mortality. Their genetic load would be entirely (or almost so) of the mutational load type, that is, due to lethal and semi-lethal genes not belonging to systems of balanced polymorphism and so being maintained by recurrent mutation. This problem will be discussed in full elsewhere[4].

We want to emphasize that the results of the present work refer exclusively to the populations under investigation. No generalization can be made uncritically.

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Origin of Expressed Mutations in *Schizophyllum commune*

PREVIOUS investigations on tetrapolar sexuality in Basidiomycetes[1-3] have shown that morphologically aberrant sectors arise in surprisingly high frequency in the common-A heterokaryons of *Schizophyllum commune*. These heterokaryons are established whenever matings are made of two strains which share common factors at one of the two complex loci governing incompatibility, the A factor[1]. On further isolation these sectors are capable of growing into homokaryotic mycelia of a limited number of phenotypes, and in the majority of cases each type can be shown to be the result of a single-gene mutation[4]. No such occurrence of visibly expressed mutations has ever been observed within homokaryons. Among the hypotheses which have been proposed to account for the origin of these expressed mutations are[3]: (a) increased mutagenesis within the heterokaryon at a few specific loci; (*b*) increased mutagenesis at all loci followed by selection of the few specific types encountered; (*c*) no increase in mutagenesis, but selection of a few specific types of low-level, spontaneous mutation.

The present report offers evidence which tends to support the last hypothesis. By means of a null-point method, similar to that employed by Luria and Delbruck in their classic researches on mutation in bacteria[5], the mutation-rates of three common-*A* heterokaryons and of their component homokaryons have been determined. This technique makes possible a comparison of the 'basic mutability', uncomplicated by the subsequent phenomena of nuclear migration and differential multiplication.

A number of series of replicate microcultures of each mycelial type were grown on a limiting concentration of nutrient (10^{-3} the concentration of a standard glucose–peptone medium[1]). At the end of a suitable period of growth, usually 21 days, after which time 10^{6}–10^{7} cells were present, each microculture was individually macerated in a Waring Blendor. The fragments were plated out on complete medium, and the resulting colonies were visually screened for the presence of mutant types. On the assumption that the mutations were distributed according to Poisson's Law[5], the fraction of cultures within any one series which contain no mutants is equal to e^{-m}, where m is the average number of mutations per culture. The rate of mutation per nucleus per nuclear cycle is then equal to m divided by the average number of nuclei per culture, N. The value of N was calculated from viable fragment counts by means of a correction factor which expressed the ratio of a microscopically measured number of cells and the viable fragments surviving the maceration thereof.

It was seen, first of all, that mutations could indeed be detected in homokaryotic microcultures as well as in heterokaryons. This finding has previously been reported only sporadically[3,4]. Secondly, the rate of mutation within any one heterokaryon, about 10^{-7} per nucleus per nuclear cycle, was not appreciably higher than the rate within either of the component homokaryons. Thirdly, the various series, homokaryotic as well as heterokaryotic, showed no marked differences in the spectrum of mutations obtained; thus, fourteen more or less distinct morphological classes were isolated, and almost all classes were recoverable from any one mycelial type. Twenty-five such mutants, of various types and derived from both homokaryotic and heterokaryotic mycelia, were crossed to wild type; the mutant character segregated as a single-gene trait in every case. These experiments were repeated using 5- and 10-day-old cultures. The proportion of mutant-bearing cultures decreased in accordance with the smaller total cell population per culture, but the estimations of the mutation-rate were substantially the same as for the 21-day-old cultures. Hence, the mutation-rate appears to remain constant for at least the period 5–21 days. Although differences in expressed mutation have been observed between differently constituted heterokaryons[2], no difference in basic mutability was found between the three heterokaryons studied here. Furthermore, increasing the degree of isogenicity between the component strains of a heterokaryon (to the extent of 10 back-cross generations) had no effect on the mutation-rate or spectrum. A summary of the principal results is given in Table 1.

Table 1. MUTATION-RATES COMPUTED FROM VARIOUS VALUES OF m AND N

Strain	Age	Mean N	m	Rate of mutation ($\times 10^{-7}$)
699	5 days	264,000	0·0516	2·0
*E*602	,,	229,000	0·0622	2·7
(699+*E*602)	,,	762,000	0·1737	2·3
699	10 days	—	0·3852	4·2*
*E*602	,,	917,000	0·1310	1·4
(699+*E*602)	,,	1,000,000	0·1656	1·7
699	21 days	4,055,000	0·5990	1·5
*E*602	,,	4,192,000	0·4763	1·1
447	,,	—	0·2891	0·6†
(699+*E*602)	,,	4,030,000	0·7310	1·8
(699+447)	,,	6,615,000	0·4946	0·8
*B*1	,,	8,500,000	0·3293	0·9
*B*9	,,	8,858,000	0·4447	0·5
(*B*1+*B*9)	,,	2,667,000	0·9163	3·4

* Estimated on the basis of $N = 917{,}000$.

† Estimated on the basis of $N = 4{,}112{,}000$.

To test the possibility that these results were due to the decidedly suboptimal conditions of growth obtaining within the microcultures, agar-plate mycelia of the above series were established on complete medium. Plugs from the older regions were excised, macerated, and examined for the presence of mutant fragments. It was found that mutant fragments could be recovered from homokaryons under these conditions as well. In general, mutant nuclei were present in heterokaryons in greater abundance than in homokaryons, but since there was considerable variation between individual samples of a particular mycelial type, further investigations of a more extensive nature are necessary to confirm this point.

It would appear from these preliminary results that the rate of mutation of any particular nuclear type is basically the same whether that nucleus exists (*a*) homokaryotically or (*b*) heterokaryotically in association with another nucleus having the same *A* factor. Thereafter, however, the differing mycelial conditions in homokaryons and heterokaryons operate to promote the phenotypic expression of the mutations within the latter mycelial type only. Whether the stage of subsequent mutant development which is affected is the multiplication of mutant nuclei or the phenotypic expression of existing mutant nuclei is not quite clear. The recovery of considerable numbers of mutant nuclei from homokaryons after maceration, even though the intact mycelia showed no evidence of mutant sectors, suggests that the phenotypic expression of existing mutant nuclei can in some way be suppressed or masked in the homokaryon. Experiments will be carried out shortly to determine in reconstituted systems the extent of selection against mutant nuclei in homokaryons, as well as the factors in the mycelial environment which significantly affect the degree of selection.

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[1] Papazian, H. P., *Bot. Gaz.*, 112, 143 (1950).
[2] Raper, J. R., and San Antonio, J. P., *Amer. J. Bot.*, 41, 69 (1954).
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[4] Raper, J. R., and Miles, P. G., *Genetics*, 43, 530 (1958).
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[6] Roshal, J. Y., Ph.D. Dissertation, University of Chicago (1953)

FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, January 9

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. G. H. Metson: "Recent Research in Thermionics".

Illuminating Engineering Society (at the Federation of British Industries, Tothill Street, London, S.W.1), at 6 p.m.—Mr. H. R. Ruff, Mr. H. E. Bellchambers and Mr. G. K. Lambert. "Modern Aids to Lighting Design—Computer Techniques".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Emil Schulthess: "Portrait of Antarctica".

Tuesday, January 10

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Scientific Papers.

Institution of the Rubber Industry (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p.m.—Mr. S. C. Stokes: "Rubber Bonded Non-Woven Fabrics"; 7 p.m.—Mr. R. L. Zapp: "Recent Developments in Butyl Rubber".

Society of Chemical Industry, Plastics and Polymer Group (joint meeting with the Chemical Engineering Group, at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. G. A. Rawcliffe and Dr. T. Love: "Some Industrial Uses of Plastics".

Royal Society of Medicine, Psychiatry Section (at 1 Wimpole Street, London, W.1), at 8 p.m.—Discussion on "Implications on Recent Genetic Research for Psychiatry". Speakers: Prof. L. S. Penrose, F.R.S. and Prof. P. Polani.

Wednesday, January 11

Society of Chemical Industry. Microbiology Group (joint meeting with the Society for Applied Bacteriology, in the Barnes Hall of the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 2.15 p.m.—Meeting on "Quantitative Microbiology".

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Annual General Meeting. 7.15 p.m.—Dr. G. F. Taylor: "Multi-layer Switching Devices".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. C. B. Frisby: "Ergonomics—Fitting the Job to the Worker".

Women's Engineering Society (at "Hope House". 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—"Ground Effect Machines". Speaker from Britten-Norman, Ltd.

Thursday, January 12

Royal Society of Arts, Commonwealth Section (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Captain John Noel: "Photography and Exploration in the Himalayas".

Institute of Metals, Metal Physics Committee (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. E. Hilliard (U.S.A.): "Metallurgical Research at High Pressures".

Chemical Society (in the Lecture Theatre, The Royal Institution, Albemarle Street, London, W.1), at 7.30 p.m.—Prof. R. D. Haworth, F.R.S.: "Some Problems in the Chemistry of the Gallotannins" (Pedler Lecture).

Radar and Electronics Association (at the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 7.30 p.m.—Mr. G. Stannard: "Programme Circuits on Telephone Plant".*

Friday, January 13

University of London (at Imperial College of Science and Technology, City and Guilds College, Exhibition Road, London, S.W.7), at 4.30 p.m.—Prof. C. J. D. M. Verhagen: "Education and Research into Instrumentation in the Technological University of Delft".*

Saturday, January 14

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss M. V. Thornton: "Indians of Canada".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Experimental Officer (with a School Leaving Certificate with passes in three subjects at higher level, including two science subjects; a Higher National Certificate or equivalent) to be responsible for preparative work in a research programme concerned with biochemical and developmental genetics—The Secretary, Poultry Research Centre, Agricultural Research Council, King's Buildings, West Mains Road, Edinburgh 9 (January 14).

Lecturer or Assistant Lecturer (Grade B) in Electronics; and an Assistant Lecturer (Grade B) in Physics—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff (January 14).

Research Assistant (with a suitable veterinary qualification or a degree in agriculture or natural science) in the Department of Animal Husbandry and Veterinary Hygiene, for studies in poultry husbandry—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (January 14).

Lecturer (preferably with previous experience in the application of computers in statistics and/or operational research) in the Electronic Computing Laboratory—The Registrar, The University, Leeds 2 (January 20).

Senior Lecturer or Lecturer in Electrical Engineering—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee, Scotland (January 21).

Senior Lecturer in Geology, and a Lecturer in Geology at the University College of the West Indies—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 27).

Senior Lecturer (preferably with research interest in the application of mathematics to some engineering subject) in charge of the Department of Engineering Mathematics—The Secretary, The Queen's University, Belfast, Northern Ireland (January 30).

Assistant Lecturer in Physics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (January 31).

Senior Lecturer (with research and teaching experience in the fields of cardiovascular physiology and respiratory physiology) in Physiology at the University of New South Wales—The Agent-General for New South Wales, 56 Strand, London, W.C.2, and the Bursar, University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia, marking envelope "University Appointment" (January 31).

Assistant Lecturer or Lecturer in the Department of Pure Mathematics—The Registrar, The University, Liverpool (February 3).

Lecturer or Assistant Lecturer (with a particular interest in sedimentary petrology) in Geology—The Secretary and Registrar, The University, Southampton (February 3).

Senior Lecturer (familiar with modern methods in clinical biochemistry, and preferably holding a medical qualification) in Clinical Biochemistry at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 4).

Imperial Chemical Industries Research Fellows in Physics, Chemistry, Biochemistry, Engineering, Metallurgy and Pharmacology or any related subjects—The Registrar, The University, Liverpool, quoting CV/N (February 11).

Lecturer or Assistant Lecturer (with a good honours degree in engineering, mathematics or physics, together with suitable experience in hypersonics or noise and vibration theory) in either Hypersonics or Noise and Vibration Theory in the Department of Aeronautics and Astronautics—The Secretary and Registrar, The University, Southampton (February 13).

Hazel Clifford Postgraduate Student, for research in medicine or in a scientific topic concerned with the advancement of medical knowledge—The Secretary to the Scholarships Committee, University of London, Senate House, London, W.C.1 (February 14).

Imperial Chemical Industries Research Fellows in various branches of Chemistry, Chemical Engineering, Engineering, Technology, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14).

Turner and Newall Research Fellows in Engineering, Inorganic Chemistry, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14).

Senior Lecturer or Lecturer (with a higher degree, preferably a doctorate, with special qualifications in some branch of physical chemistry) in Physical Chemistry at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 15).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Engineering, Pharmacology, Physics or Chemical Technology (Chemical Engineering)—The Secretary, The University, Edinburgh (February 16).

Lecturer (preferably with training and/or publications in the field of human and economic geography) in Geography at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 25).

Lecturer (with a Ph.D. or a degree in veterinary science with postgraduate experience in protozoology) in Protozoology within the Department of Parasitology, University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 28).

Imperial Chemical Industries Research Fellow in Chemistry, Biological Chemistry, Engineering, Pharmacology, Physics or in a related subject—The Registrar, The University, Bristol 8 (March 1).

Departmental Demonstrator in Biochemistry—The Administrator, Department of Biochemistry, The University, South Parks Road, Oxford (March 4).

Senior Lecturer (with a science degree and research experience to Ph.D. or comparable level, some experience in mineral and rock analysis and the ability to do fundamental research) in Geology (Geochemist) at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 31).

Abstractor (with a sound knowledge of German and Dutch and the ability to abstract in English from these languages, Scandinavian and/or Spanish and Portuguese an added qualification, and preferably a knowledge of microbiology and/or botany)—The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey.

Agricultural Chemist (with a good honours degree in agricultural chemistry, with some postgraduate experience, preferably in the tropics) in Sierra Leone, to advise the Director of Agriculture on specific problems, and for investigational studies in the field, including the laying-out of field trials and their statistical analysis and interpretation—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/15/04.

GRADUATE ASSISTANTS (candidates wishing to proceed to the M.Sc. in mathematics) IN THE DEPARTMENT OF MATHEMATICS—Head, Department of Mathematics, Essex College, Assumption University of Windsor, Windsor, Ontario, Canada.

GRADUATE ASSISTANTS (candidates wishing to proceed to an M.Sc. in physics) IN THE DEPARTMENT OF PHYSICS—The Chairman, Department of Physics, Essex College, Assumption University of Windsor, Windsor, Ontario, Canada.

READER IN APPLIED NUCLEAR PHYSICS—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH FELLOW IN THEORETICAL PHYSICS, NUCLEAR PHYSICS or SOLID STATE PHYSICS—The Secretary Research Institute for Fundamental Physics, Kyoto University, Kyoto, Japan.

SCIENTIFIC ASSISTANT (female, preferably graduate with microbiology as one subject and an interest in virology)—The Director, Public Health Laboratory, Priorsdean Hospital, Milton Road, Portsmouth.

SCIENTIFIC OFFICERS (MINERAL DRESSERS) (with a good honours degree in mineral dressing or mineral engineering, and preferably not over 35 years of age, with some experience in industry) IN THE GEOLOGICAL SURVEY DIVISION of the Federal Ministry of Mines and Power, Nigeria, to undertake research on mineral dressing, particularly in connexion with the recovery of minerals from alluvial concentrates in the Plateau tinfields—The Appointments Secretary, Federal Public Service Commission, Nigeria House, 9 Northumberland Avenue, London, W.C.2, quoting J.2/8.

SENIOR BOTANIST (with a first- or second-class honours degree in botany or equivalent qualification and at least 15 years research experience and a good record of published work. Proven ability to plan and supervise research programmes and experience with tree crops, preferably in the tropics, is also desirable), to take charge of the Botany Division (comprising plant breeding and plant physiology sections) of the West African Cocoa Research Institute, Ghana—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.197/200/028/01.

SENIOR LABORATORY TECHNICIAN (with a knowledge of chemistry)—The Assistant Secretary. Royal Veterinary College, Royal College Street, London, N.W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Pure and Applied Chemistry, Vol. 1, No. 1. (The Official Journal of the International Union of Pure and Applied Chemistry.) Pp. 186. Published irregularly, four issues per volume. Subscription per volume including postage—U.K. £6; U.S.A. 18 dollars. (London: Butterworth and Co. (Publishers), Ltd., 1960.) [1210

Department of Scientific and Industrial Research: Road Research Laboratory. Road Note No. 29: A Guide to the Structural Design of Flexible and Rigid Pavements for New Roads. Pp. i+27. (London: H.M. Stationery Office, 1960.) 2*s*. net. [1210

Building Research Station Digest. Second Series. No. 2: Questions and Answers. Pp. 2. 4*d*. No. 3: Working in Winter or Bad Weather. Pp. 4. 4*d*. (London: H.M. Stationery Office, 1960.) [1210

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General Register Office. The Registrar General's Quarterly Return for England and Wales, No. 446 (2nd Quarter, 1960): Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, quarter ended 30th June, 1960. Pp. 28. (London: H.M. Stationery Office, 1960.) 2*s*. net. [1410

Department of Scientific and Industrial Research. Waste or Wealth? By Clement Brown. Pp. 27. (London: H.M. Stationery Office, 1960.) [1410

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Socrates on the Health Service. (Dialogues of Today from *The Lancet*.) Pp. 102. (London: The Lancet, Ltd., 1960.) 5*s*. net. [1410

Federation of British Industries. Overseas Trainees in United Kingdom Industry. Pp. 16. (London: Federation of British Industries, 1960.) 6*s*. [1410

University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1958–59. Pp. 58. (Cmnd. 1166) (London: H.M. Stationery Office, 1960.) 4*s*. 6*d*. net. [1410

Bulletin of the British Museum (Natural History). Entomology. Vol. 9, No. 9 Sur les Pselaphides (Coleoptera) de l'Inde Septentrionale Par René Jeannel. Pp 401–456. 22*s*. Vol. 9, No. 10: On the Synonymy of some *Zygaena* Species, with Descriptions of a New Species and Subspecies from Morocco, Lep., Zygaenidae. By Hugo Reiss and W. Gerald Tremewan. Pp. 457–468+plates 22–25. 9*s*. Vol. 10, No. 1: A Taxonomic Study of the Larvae of West African Simuliidae (Diptera · Nematocera), with comments on the Morphology of the Larval Black-Fly Head. By R. W. Crosskey. Pp. 1–74. 21*s*. Historical Series. Vol. 2, No. 5. Darwin's Notebooks on Transmutation of Species. Part 4: Fourth Notebook (October 1838—10 July, 1839). Edited with an Introduction and Notes by Sir Gavin de Beer Pp. 151–183. 15*s*. (London: British Museum (Natural History), 1960.) [1410

Chesterfield Tube Co., Ltd. Fabrication of Beryllium Tubes and Sections (a New Structural Metal with Unique Properties). Pp. 16. (Chesterfield: The Chesterfield Tube Co., Ltd., 1960.) [1410

Educational Productions Limited. Filmstrip No. CX 6290: Seed Germination. 23 frames. 27*s*. 6*d*. Notes for Filmstrip No. CX 6290 by Alan Dale. Pp. 8. Filmstrip No. CX 6332: Grasses. 27 frames. 27*s*. 6*d*. Notes for Filmstrip No. CX 6332 by Dr. John H. Elliott. Pp. 13. Filmstrip No. C 6375: Flowers of Moorland and Bog. 33 frames. 27*s*. 6*d*. Notes for Filmstrip No. C 6375 by Dr. John H. Elliott. Pp. 15. (East Ardsley, Wakefield: Educational Productions, Ltd., 1960.) [1410

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Report of the Advisory Committee on the Examination of Steam Boilers in Industry. Pp. 23. (Cmnd. 1173.) (London: H.M. Stationery Office, 1960.) 1*s*. 3*d*. net. [1710

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Canada: Department of Northern Affairs and National Resources National Museum of Canada. Natural History Papers. No. 4 (June 15, 1960): A Hadrosaurian Ichnite. By W. Langston, Jr. Pp. 8. No. 5 (July 4, 1960): Keys to the Marine Fishes of Arctic Canada. By D. E. McAllister. Pp. 21. No. 6 (June 2, 1960): Some Noteworthy Accessions to the National Museum Mammal Collection. By A. W. F. Banfield. Pp. 2. No. 7 (June 13, 1960): A Giant Ultra-Abyssal *Cocculina* (Mollusca, Gastropoda) from the Argentine Basin. By Arthur H. Clarke, Jr. Pp. 4. No. 8 (August 30, 1960): The Survival of the Wood Bison (*Bison bison athabascae* Rhoads) in the Northwest Territories. By A. W. F. Banfield and N. S. Novakowski. Pp. 6. Canadian Atlantic Sea Shells. By E. L. Bousfield. Pp. v+72+13 plates. 1 dollar. Les Mammifères du Canada. Par Austin W. Cameron. Pp. 89. 50 cents. (Ottawa: Queen's Printer, 1960.) [1410

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Proceedings of the United States National Museum, Smithsonian Institution. No. 3429: A Revision of the Genus *Ogcodes* Latreille, with particular reference to Species of the Western Hemisphere. By Evert I. Schlinger. Pp. 227–336+plates 1–13. No. 3440: Assassin Bugs of the Genus *Ghilianella* in the Americas (Hemiptera, Reduviidae, Emesinae). By J. Maldonado-Capriles. Pp. 393–450. No. 3441. Welcome Mound and Effigy Pipes of the Adena People. By Frank M. Setzler. Pp. 451–459. (Washington, D.C.: Government Printing Office, 1960.) [1410

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University of Wisconsin: Agricultural Experimental Station, Madison. Bulletin No. 544 (North Central Regional Publication No. 76): Soils of the North Central Region of the United States—Their Characteristics, Classification, Distribution, and Related Management Problems. Pp. 192. (Madison: University of Wisconsin, Agricultural Experiment Station, 1960.) 2 dollars. [1410

Indian Council of Medical Research. The Nutrition Research Laboratories, Hyderabad, Deccan. Annual Report for 1958–59. Pp. iii+58. (Hyderabad: Nutrition Research Laboratories, 1959.) [1410

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The Carlsberg Foundations Oceanographical Expedition Round the World 1928–30, and previous "Dana" Expeditions. "Dana" Report No. 52: Oxycephalidae *Amphipodes pelagiques*. Par Louis Fage. Pp. 145. (Copenhagen: Andr. Fred. Høst and Søn, 1960.) Danish kr. 40. [1410

Food and Agriculture Organization of the United Nations. Picture Sheet No. 10 · The Fellowships Scheme. Pp. 2. Picture Sheet No. 11: Animal Health. Pp. 12. Picture Sheet No. 12 Good Seed Doesn't Cost, It Pays. Pp. 2. (Rome. Food and Agriculture Organization of the United Nations, 1960.) [1410

European Nuclear Energy Agency, Paris. Eurochemic: European Company for the Chemical Processing of Irradiated Fuels. Pp. 24. (Paris: European Nuclear Energy Agency, 1960.) [1710

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NIGERIA'S INVESTMENT IN EDUCATION

THE proposals of the Commission on Post-School Certificate and Higher Education in Nigeria, reviewed on page 95 of this issue, are indeed massive, expensive and unconventional. To fulfil its recommendations 600 secondary schools, 6 technical institutes, 8 teacher-training colleges and 3 universities will have to be built and staffed during the next ten years. The programme will require about 4,000 graduate teachers on short-term contracts and 1,000 university teachers. The capital cost of the universities alone will approach £20 million, and a realistic estimate of the total recurrent costs would be £50 million over the next decade. To provide sufficient graduate teachers will entail an expenditure of about £20 million over the ten-year period. The Commission makes no estimate of the capital cost of the schools, colleges and institutes; but it seems unlikely that the overall cost of implementing the proposals will be less than £150 million between now and 1970. Aid will have to be provided on an international scale.

The Commission has obviously been placed in a dilemma. A programme of this magnitude is necessary to enable Nigeria, a country with potential, rather than present, wealth, to expand economically and take its right place among the nations. But the proposals come at a time when there is considerable expansion in secondary schools and universities throughout the Commonwealth. The recruitment of sufficient teachers to 'prime the pump' of this educational programme is in itself a major project. To make it succeed, there will have to be considerable co-operation between Nigeria, Britain and the United States of America. The secondment of both school and university teachers will have to be encouraged from the English-speaking countries. Nigeria, on her part, must be willing to show a spirit of welcome to the large numbers of expatriates; to appreciate that decisions in educational matters must be independent of the changing winds of politics; and be eager to accept the advice of experts on the many problems which will arise.

In the last resort, the universities are the backbone of the country's educational system. It is important, therefore, to examine critically the Commission's recommendations as to how these institutions should be developed, governed and financed. It is proposed that there should be one large university in each of the three Regions, and that they should be financed largely from Federal funds. It is also suggested that the regional branches of the Nigerian College of Arts, Science and Technology, which are Ibadan in the west, Enugu in the east, and Zaria in the north, should become centres of university education

While the Ibadan branch could be absorbed easily by the adjacent University College, it would be more difficult for the Enugu branch of the Nigerian College to form as close links with the University of Nigeria, at Nsukka, some 40 miles away. In the Northern Region, the problem of geographical separation of the different parts of a single university is more acute. Zaria, which already has an engineering faculty as a basis for university development, is 75 miles from the Ahmadu Bello College of Arabic Studies at Kano, and is 150 miles from Vom, where considerable development of the Veterinary Research Institute is envisaged. From this aspect alone, it may be necessary for the separate units to have much greater independence than would be possible in a single university. This might also help to solve another difficulty of which the Commission has taken insufficient cognizance.

In Nigeria to-day there are strong feelings of regional loyalty. These were a large part of the driving force which has already led to a university being established at Nsukka, to the development of the College of Arabic Studies at Kano, and also to the intention of the Western Region Government to found a new university. These institutions may prefer to be financed mainly from regional funds, as they are at present, and become regional universities. The centres at Zaria, Vom and Enugu could continue to receive federal support to develop faculties of technology, agriculture and veterinary science, and would become federal universities, as University College, Ibadan, has always been. It is unlikely that the regional universities would become in any way academically inferior to the federal universities, especially if the new institutions seek the sponsorship of well-established universities overseas. The relationship between the University of London and University College, Ibadan, has proved invaluable in establishing high academic standards. The federal and regional universities would be complementary; the more expensive medical and technological faculties being in the Federal institutions, while the regional universities could develop characteristics of the particular needs and traditions of their regions. Both types would be autonomous, and both would admit any suitably qualified student.

Of the 2,000 students who will graduate annually, there will be 800 teachers, 600 technologists, and 200 doctors; 200 will have agricultural or veterinary qualifications and 200 will have commerce degrees. The Commission deals with the training of these groups in detail, except for the technologists, who form 30 per cent of the total output. It would be particularly helpful to know what proportions should be trained in metallurgy, chemical, civil, mechanical, and electrical engineering, mining and other technologies, so that an estimate can be made of the size of the departments which are needed in the universities. Until a more detailed survey of man-power needs has been made, it is difficult to make these estimates accurately.

One-half of the annual output of graduates must go into either teaching, agricultural, or veterinary appointments. At present these are the professions which receive the fewest numbers of graduates. It seems unrealistic to suppose that sufficient undergraduates will elect to prepare for the B.A. (Education) degree, or for agricultural or veterinary degrees, unless there is some substantial inducement. Since the success of the whole programme depends so much on an adequate flow of graduates into these professions, more detailed recommendations on this point, based on the experience of members of the Commission, would be helpful.

There is one danger which the report does not stress sufficiently strongly. This is that the universities, in expanding rapidly to meet a need for trained men and women, may tend to become centres where knowledge is disseminated rather than places where knowledge is acquired. This is simply to say that great stress must be laid on research. This is particularly the case in arts, science and technological faculties offering general courses, which will be much less specialized than those for single honours degrees. To maintain a balance and a high level of scholarship good research facilities must be provided. Only with these provisions will expatriates and Nigerians of high calibre be attracted to staff the universities, and will Nigerians be trained to replace gradually the expatriates. For some years to come, the burden of this training must fall on University College, Ibadan. In connexion with research facilities, a most welcome suggestion is that each university should have a Research Institute of African Studies attached to it.

The proposal to found a new university at Lagos, which would have two main constituent schools, Commerce and Business Administration and also Economics and Social Sciences, is a sound one. As the Commission points out, in an under-developed country, the universities must accept the responsibility of training accountants, bankers, transport executives, etc., because facilities for training 'on the job' are so lacking.

The Commission has faced squarely three other aspects of the educational programme. These are the provision of sufficient secondary school places, particularly in sixth forms; creating a virtually new teacher-training programme; and expanding technical institutes which will give training at an intermediate level. The problems which may arise here will result mainly from the rapid growth and sheer size of these projects.

These aspects are quite as important as the university development, for the whole is an integrated programme. The Commission has been bold and imaginative in its approach to Nigeria's educational needs. For the country's proper development ways must be found to make the plan succeed as a whole. It is sincerely to be hoped that international aid on a large scale will be forthcoming to help this "awakening giant of Africa", as Nigeria has been so aptly called.

COTTON IN INDIA

Cotton in India

A Monograph. By B. L. Sethi, S. M. Sikka, R. H. Dastur, P. D. Gadkari, R. Balasubrahmanyan, P. Maheshwari, N. S. Ranga Swamy, and A. B. Joshi. Pp. xiv+474+30 plates. (Bombay: Indian Central Cotton Committee, 1960.) Rs. 30.; 45*s*.

THIS is the first of four volumes of a monograph, "Cotton in India", in which the Indian Central Cotton Committee is gathering together all available information in the domains of cotton culture, technology and marketing. The present volume is mainly concerned with research work in India, but gives a creditable, if not always critical, survey of the work done in other countries. The eight chapters cover the subject-matter as follows: history of cotton (B. L. Sethi); climate and soils (S. M. Sikka and R. H. Dastur); taxonomy (P. D. Gadkari); morphology (R. Balasubrahmanyan); embryology (P. Maheshwari and N. S. Ranga Swamy); breeding (S. M. Sikka and A. B. Joshi); genetics (S. M. Sikka and A. B. Joshi).

There is great need for a monograph of this kind, since cotton cultivation in India occupies an area of about 20,000,000 acres—about six per cent of the whole arable area. There are extensive and well-planned schemes of research in all the widely scattered cotton-growing areas. Large numbers of young and enthusiastic workers are without facilities for consulting the relevant literature. An encyclopædic work of this kind, for the most part soberly and factually written, will be of great use not only to Indian workers but also to those in other countries, especially as the authors have avoided the parochial attitude of over-emphasizing Indian contributions, which indeed often represent pioneer work of great merit.

On the whole the authors have done their task well, and have produced a work of great value. There are a few errors of emphasis and interpretation; for example, the term genetical erosion is used in a wrong sense. It does not, as Sikka and Joshi imply, mean the breakdown which occurs in the second generation of interspecific crosses. It means the impairment of primitive adaptive complexes through extensive substitution of genes in the processes of selection or domestication. The first exploitation of the back-cross method is ascribed to Harland and Pope, instead of Harlan and Pope.

It is only to be expected that exuberant optimism will at times lead authors to express favourable opinions on future possibilities which are scarcely justified by existing scientific information. Thus, on p. 38, the reader is given to understand that sea island cotton can be grown successfully on the west coast (Kerala). It is, however, well known that the establishment of a sea island industry in this region would encounter almost insuperable obstacles of climate, soils, pests and disease. Its ultimate success is therefore doubtful. The main tasks of the breeders in India are first to increase yields to a level comparable with other cotton-growing countries, and second, to breed cottons which will spin good 40's. At present such cottons do not exist except perhaps occasionally in small experimental lots.

The book is well produced and relatively cheap. The index is poor, and there is no author index.

S. C. Harland

MINERAL PROCESSING

Proceedings of the Second United Nations International Conference on the Peaceful Uses of Atomic Energy

Held in Geneva, 1 September–13 September, 1958. Vol. 3: Processing of Raw Materials. Pp. ix+607. (Geneva: United Nations; London: H.M. Stationery Office, 1958.) 64 Swiss francs; 106*s*.; 15 dollars.

THIS volume is a collection of seventy-eight papers, each dealing with some aspect of the technology of winning and processing the ores of metals of interest in the field of atomic energy. A group of three papers deals with the methods of mining used for obtaining uranium ores in North America. Five are concerned with mathematical aspects of evaluating ore deposits and nine with instrumental methods of detecting and estimating radioactive materials, including potassium salts. The rest of the volume is concerned almost entirely with chemical aspects of mineral processing and physical methods of analysis for uranium, thorium and beryllium. There is also a valuable review of the chemical methods available for estimating uranium.

Very little work is reported on physical methods of ore concentration, so presumably the conventional techniques have proved adequate. An automatic method of sorting uranium-bearing rock using the radioactivity of desirable pieces to identify them is described, however, and there is an account of a normal type of process for separating the constituents of a beach sand containing monazite. There has also been limited interest in flotation as a means of concentrating minerals relevant to the Conference except in special cases. One of these concerns a lode deposit of monazite in South Africa which could not be treated by electrostatic and magnetic methods and another is, of course, the difficult problem of the separation of beryl from the other silicates which accompany it in Nature. The paper dealing with the latter topic shows with particular emphasis that valuable as the information presented at this Conference may be, there is much that is not given. Thus a large amount of data on the use of carboxylic acids, such as oleic acid, for separating beryl is given, followed by a table showing very much better results. These are said to have been obtained using a "special procedure"; but whether this means that the same reagents were used in a different way or that other reagents are better than those described in the paper is not clear.

Pretreatment of uranium ores before dissolving out the uranium has not been necessary in most cases, and is scarcely likely to be economically possible in the near future. However, a large amount of the known potential uranium reserves occurs as very low-grade ore, and three papers deal at least in part with the flotation of uranium minerals. The most active field of work in uranium mineral processing has undoubtedly been the study of methods for dissolving the uranium and recovering it from the resulting aqueous solution. Leaching either with acid or carbonate–bicarbonate mixtures has received about equal attention, and although the chemistry of both processes is well understood there are some interesting accounts of their application to particular ores. The work designed to produce an oxidizing sulphuric acid solution suitable for use as a leaching reagent by oxidation of sulphur dioxide in the presence of manganese or ferric ions as catalyst is worthy of particular attention.

Recovery of uranium from solution is carried out by solvent extraction or ion exchange, and there is a comparison of the relative merits of the two processes in various circumstances. Considerable interest in the treatment of unclarified solutions using the resin-in-pulp method or solvent extraction of the slurry is shown by the number of papers on these subjects. This is due to the difficulty of removing the suspended solids, and one communication deals with the use of flocculating agents to assist in this process. Of interest also is the method of reducing uranium in solution to precipitate the dioxide, using hydrogen with nickel or uranium dioxide as catalyst.

Chemical methods of cracking minerals are not confined to uranium treatment, of course, but few other cases are dealt with. There are, however, papers dealing with the breaking down of thorium minerals and of zircon.

It is unfortunate that the volume contains no index and that few of the papers include a summary. In spite of this, however, the book should prove valuable to anyone wishing to gain an idea of recent advances in the hydrometallurgical processing of minerals. A. R. BURKIN

ELECTROMAGNETISM AND RELATIVITY

Les Principes de la Théorie Électromagnétique et de la Relativité

Par Prof. Marie-Antoinette Tonnelat. Pp. 394. (Paris: Masson et Cie., 1959.) 5,000 francs.

THIS text-book is divided into three parts, dealing with electromagnetism, special relativity and general relativity. The section on special relativity is the largest and the most complete part of the book. It sets out the historical development of the ideas and concepts which led, in the end, to the special theory, and it traces very carefully the interplay between the theoretical concepts and the few decisive experiments during the course of this development.

The main factors on the theoretical side were very straightforward. First, Newton's equations of motion and the Galilean invariance which resulted implied that no (inertial) reference system had preference over any other system moving uniformly relative to the first. Then, later, the nineteenth-century ideas on the propagation of light appeared to single out one absolute reference system, which was specified by the ether. Later again, it was seen that Maxwell's equations were not Galilean invariant, and therefore they, too, supported the idea of the absolute reference system. Thus it seemed that all that was needed to determine absolute motion was a few accurate optical experiments. However, the culmination of these experiments was the famous interferometer work of Michelson and Morley, which gave the nul result.

This story is well told, with adequate digressions to deal with related matters of interest. For example, the relation is pointed out between the experiments of Fizeau, Hoek, and others on the propagation of light in moving media, and the experiment of H. A. Wilson on the effects of moving a dielectric in a magnetic field. In addition, the mathematical apparatus of special relativity is developed in a very complete treatment, and numerous applications of the special theory are given.

Unfortunately, the book could not contain an account of the recent work of Terrell and of Penrose[1] on the Lorentz–Fitzgerald contraction. A fast-moving object, it is well known, is contracted in the direction of its motion by a factor $(1-v^2/c^2)^{1/2}$. Thus, for example, a fast-moving cube becomes a rectangular parallelepiped with the shortest principal axis along the direction of motion. However, Terrell and Penrose have pointed out that this is not what an observer would see. For example, an observer at right angles to the line of flight of the cube would see the rear face of the cube as well as the face nearest to him. The result is that the cube appears to the observer to be rotated, but undistorted. (This is true provided the cube subtends a small angle at the observer.) The Lorentz contraction is nevertheless still valid. If (v/c) is close to unity, the face nearest the observer has shrunk to zero width, but the observer sees the rear face at full size.

The section on electromagnetism contains a rather formal treatment of Maxwell's theory; for example, the electrostatic properties of conductors are derived before there has been any discussion of the presence of electrons in conductors. Indeed, the main purpose of this section appears to be to emphasize that electromagnetic phenomena can be described entirely by fields rather than by action at a distance.

The final section is that on general relativity. Here there is a good mathematical treatment, with several interesting worked examples, and an adequate physical discussion. However, it is at this point that one would expect some discussion of fairly new ideas and developments. Some comment might be expected on whether we can hope to detect gravitational waves, what is the present status of theories of the expanding universe, whether we should expect there to be anti-galaxies, and whether the latter have reversed gravitational interaction with ordinary galaxies. Discussion of some of these topics might perhaps have been more rewarding than the account of the unified theories of gravitation and electromagnetism with which the book concludes.

J. HAMILTON

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[1] Penrose, R., *Proc. Camb. Phil Soc.*, **55**, 137 (1959). Terrell, J., *Phys. Rev.*, **116**, 1041 (1959).

ESSENTIALS OF LOGISTICS

A Précis of Mathematical Logic

By J. M. Bocheński. Translated from the French and German editions by Otto Bird. (Synthese Library.) Pp. ix+100. (Dordrecht: D. Reidel Publishing Company, 1960.) 13.75 D. fl.

ONE of the most remarkable features of twentieth-century thought is the immense creative effort of Polish scholars in the field of mathematical logic. In this, of course, they are by no means alone, since (among others) Italy, Great Britain and the United States have contributed vital elements to the growing structure. Nevertheless, there is something intrinsically majestic about the Polish work, both in the profundity of research, and in the wide range of studies. Having enriched what they have touched, they have become scholars in analysis and artists in symbolism.

This means that, sooner or later, it becomes desirable to 'tidy up', that is, to tabulate with extreme formality the propositions and theorems which the power of the new 'logistik' has produced. The result is the slender volume before us, a masterpiece of condensation and taxonomy. A touch so appropriate to a book of this character is that the introduction starts with paragraph zero, so that "Fundamentals" begin with paragraph 1, and onwards; the decimal notation is correspondingly simplified throughout.

The arrangement is: (1) "General Principles"; (2) "The Logic of Sentences"; (3) "The Logic of Predicates and Classes"; (4) "The Logic of Relations"; (5) "Varia". At the end are a table of logical signs and an exceptionally full bibliography. This latter will be welcomed as a reinforcement of the literature listed from time to time in *The Journal of Symbolic Logic*.

A further aid to the student is a short summary, at the conclusion of each section, dealing with the history and literature of the particular aspect under discussion, for example, laws and rules, predicates, the theory of identity, syllogistics and so on. It is interesting to note that the first of these originated with Husserl's distinction; Aristotle thought of his theorems as laws, whereas to the stoics and scholastics they were rules.

There is a clear account of the newest branch of the subject, namely, the logic of relations, which is capable of extension over perhaps the whole of knowledge. Even at the present early stage of development its fundamental connexion with geometry begins to appear.

The value of this précis lies in its power to bring the main results of logistics into exact focus, so that they can be seen both in derivation from classical principles and in terms of contemporary notations. Denuded of verbiage, it is remarkable how many of them were known to the Greek philosophers and to medieval savants. Yet the modern discipline (including in particular the many-valued logics) is of incomparably greater force, as well as possessing enhanced importance in advanced mathematical education.

F. I. G. RAWLINS

CATALYSIS

Catalysis, Vol. 7

Oxidation, Hydration, Dehydration and Cracking Catalysts. Edited by Paul H. Emmett. Pp. vi+378. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 108*s*. net.

THE publication of this final volume of a major work dealing mainly with industrial catalytic reactions will be welcomed by the many chemists who are interested in this rapidly moving field; and Prof. Emmett and the individual contributors are to be congratulated on making available, in a convenient form, summaries of the very large mass of material, having largely an industrial bias but relating also to some academic applications of catalysis, which lies scattered and difficult of access in the patent and journal literature.

In the first chapter of this volume, L. B. Ryland, M. W. Temele and J. N. Wilson, of Shell Development Co., deal with the use of modern fluidized and other finely divided catalysts for the cracking of crude petroleum into lighter oils. This is followed by a long section on catalytic hydration and dehydration, by M. E. Winfield, of the Commonwealth Scientific and

Industrial Research Organization, who has himself been responsible for much work on this subject. The remainder of the volume has been written by J. K. Dixon and J. E. Longfield, both of the American Cyanamid Co., who give, in successive chapters, an account of the partial oxidation of hydrocarbons, mainly on catalysts of the vanadium pentoxide or metallic platinum types, detailed attention being given to the theoretical aspect of these reactions. This is supplemented by two further chapters on the mechanism of catalytic oxidation generally and by a rather disappointingly short section on miscellaneous catalytic oxidations.

The spreading of the publication of the complete work over some six years (the first volume appeared in 1954) has necessarily rendered some of the volumes to some degree out of date and incomplete; but this is inevitable in a rapidly moving subject, and Prof. Emmett has probably acted wisely in publishing the material as it was received. The now complete work is to be strongly recommended as a necessary acquisition both by technical and academic libraries and by individual chemists. It will be found to be an exhaustive source of reference in almost all branches of modern catalysis, and should be particularly useful as a foundation for further research.

E. B. Maxted

EVOLUTION

Implications of Evolution

By Dr. G. A. Kerkut. (International Series of Monographs on Pure and Applied Biology. Division: Zoology. Vol. 4.) Pp. x+174. (London and New York: Pergamon Press, 1960.) 30*s.* net.

DR. KERKUT begins his book with seven 'assumptions' which he thinks are implied in any acceptance of the theory of evolution. The first three of these are that non-living things gave rise to living material, that this happened only once, and that all living things—viruses, bacteria, animals and plants—are interrelated. Even if we read conclusions in place of assumptions, it is by no means true that all or even a majority of the biologists who believe in evolution would accept any of these as necessarily true or as implied in their belief. Evolution is the theory that in the history of life change has taken place by 'descent with modification', and the study of the course of change is phylogeny, not the theory of evolution. If we accept evolution, it by no means necessarily follows that phylogeny is monophyletic, and certainly not that any conclusions about the origin of life are implied.

Dr. Kerkut's aim in the main part of his book is to show that these 'assumptions' are ill founded, especially that we have no good reason to believe that the viruses, bacteria, rickettsias and higher organisms are related, or that the invertebrates, and even the vertebrate classes, are monophyletic. Though our views on these questions have little bearing on our belief in evolution, they are certainly questions of interest, and it is good to have them discussed by one who disagrees with widely held views. But any discussion of such questions, if it is to carry weight, must be on a fair consideration of all the relevant evidence, and I cannot agree that Dr. Kerkut gives us that. He does not discuss all the evidence, and not always the parts of the evidence that seem weightiest to most biologists. To take one example, in considering the morphology of the cell as indicating that all animals are related, he mentions only the mitotic figure—itself one might think strong enough evidence, though he does not agree—and says nothing of the rest of cell morphology—the visible and submicroscopic structure of the cell and nuclear membranes, Golgi apparatus, mitochondria, etc. It is on these many similarities, together with other evidence, that most biologists think that the cell cannot have been evolved more than once.

Other objections to the argument of the book might be raised, but to me the most general criticism is that evidence that bears against its thesis is often not discussed. As Darwin so well realized, one is much more likely to convince by over-stating one's opponent's arguments and answering them, rather than by passing them by without discussion. On these grounds the arguments of this book cannot, in my view, be thought conclusive. It is well produced; misprints, though they occur, are not especially numerous.

G. S. Carter

ZOOLOGICAL LITERATURE FOR 1957

The Zoological Record

Vol. 94: Records of Zoological Literature relating chiefly to the year 1957. Edited by G. Burder Stratton. Pp. 1842. (London: The Zoological Society of London, 1960.) 160*s.*

THE orange-coloured bindings of the "Zoological Record" stand out in any library, for they have been coming out for nearly a century and now occupy many feet of shelving. The volumes have changed very little over the years, and the present one—Volume 94, referring mainly to the literature of 1957—retains almost the same format and the somewhat out-dated system of classification of the earlier numbers. Vermes, for example, is almost Aristotelian in its breadth, and includes Platyhelminthes, Nemathelminthes, Rotifera, Annelida, as well as an assortment of smaller groups. However, there are certain advantages in changing as little as possible a reference work of this nature, and the sections into which it is divided are conveniently arranged, so that any person consulting the "Record" will have no difficulty in finding the publications on any particular subject or by any author, with the least possible delay. The contents, apart from their general usefulness to all workers in the subject, are also of interest as a rough indication of trends in zoological research work, based on the number of publications in the various sections. These trends are difficult to understand, for while Insecta remains by far the biggest section, Mollusca now takes second place although its economic or medical significance is somewhat limited. Owing to difficulties of recording, the sections dealing with Amphibia and parasitic Protozoa have not been included in the present volume, but it is hoped that they will appear in the near future.

Zoologists throughout the world are greatly indebted to the Zoological Society of London for undertaking the publication of this invaluable compilation with such little delay, for most of the sections of Volume 94 were available in 1959 and three even in 1958, the year following the literature to which they refer.

Edward Hindle

RADIOACTIVE FALL-OUT IN ISRAEL RESULTING FROM THE NUCLEAR TESTS IN THE SAHARA DESERT

By Y. FEIGE, E. SHALMON and J. VARDI
Health Physics Department, Israel Atomic Energy Commission

J. R. GAT (GUTMANN)
Weizmann Institute of Science, Rehovoth

AND

Y. L. TOKATLY
Israel Meteorological Service

ON February 13 and April 1, 1960, nuclear test devices, reportedly in the kiloton range, were exploded in southern Algeria. These tests followed a prolonged period during which no nuclear tests were conducted, with the result that fall-out of short-lived fission products had decreased to a very low level. The tests offered an opportunity of observing fall-out from isolated and well-defined sources of activity in this area.

Radioactive materials resulting from these tests were first picked up by the Israel Fall-out Monitoring Network on February 17, and were repeatedly observed again during the following months. The interpretation of the resulting fall-out deposition pattern in the light of the meteorological data gives some insight into the movement of radioactive 'clouds' and their mode of deposition of activity.

Measurement of Fall-out

The Israel Fall-out Monitoring Network has been described by Gat and Gilat[1]. It consists of seven sampling stations where activity deposited on gummed tape is collected. The stations are situated throughout the country from Eilath on the Gulf of Aquaba to Upper Galilee, and cover hill and valley areas. In addition, air filtration is carried out at ground-level at Rehovoth with the aid of Staplex type air-pumps and a continuous air monitor. The β-activity accumulated on the samplers during the exposure period is counted on the third day after collection (after the decay of short-lived natural activity) and again at fixed time-intervals thereafter. The counters are standardized by the radiation from potassium-40 in natural potassium chloride, following accepted practice. The homogeneity of the deposited activity and the presence of 'hot particles' is determined by autoradiography of the ashed samples.

Fall-out Pattern and its Correlation with Meteorological Data

The results of deposition measurements from five of the stations are plotted in Fig. 1 where some of the pertinent local meteorological information is also given. Fig. 2 shows the decay of some representative samples plotted on a log activity versus log time-scale, with the reported explosion dates taken as the time origin. The decay data enables one to relate the activity peaks to the first, second or to previous nuclear explosions.

The main impact of the first nuclear explosion was detected in Israel on February 17. On this day a fairly large number of discrete hot particles was collected on the gummed tapes, resulting in a deposition of activity of about 25 $m\mu c./m.^2$ (activity on fourth day after collection).

The meteorological synoptic maps of that day show the passage over Israel of a cold front associated with an eastward moving centre of low pressure which had developed over the central Mediterranean some days before. The upper air at 500 mb. had flowed from the explosion region towards this 'low', continuing to move with it (Fig. 3). The front which coincided with the deposition of activity was more diffuse in the southern part of the country, as was reflected in the spreading out of the activity peak at the Eilath station. (Due to an oversight, the gummed tape of February 16 was not changed at Jerusalem until noon of the following day. Hence the activity shown on the tape of February 16 apparently includes that of the following day.)

On February 18, fall-out values returned to normal levels everywhere except at Eilath where the trailing edge of the cold front was still effective, and at Jerusalem where one single 'hot' particle was collected. (It cannot be established with certainty whether this single particle is due to some residual activity which remained aloft or whether this is a blow-over particle from material deposited on the previous day.)

During February 20–21 and again on February 24–25 anticyclonic cells, moving in a north-easterly direction from the Western Desert, passed over the Levant. Both occurrences were associated with some deposition of fall-out. Backtracking of the air masses shows that their probable source was low-altitude air (< 3 km.) near the test site. Air circulation had been stationary over the whole of North Africa during this period. From these more or less stationary air masses over North Africa 'bubbles' were detached, in conjunction with the formation of anticyclonic cells, which slowly moved north-eastward into the Mediterranean and over Israel (Fig. 4).

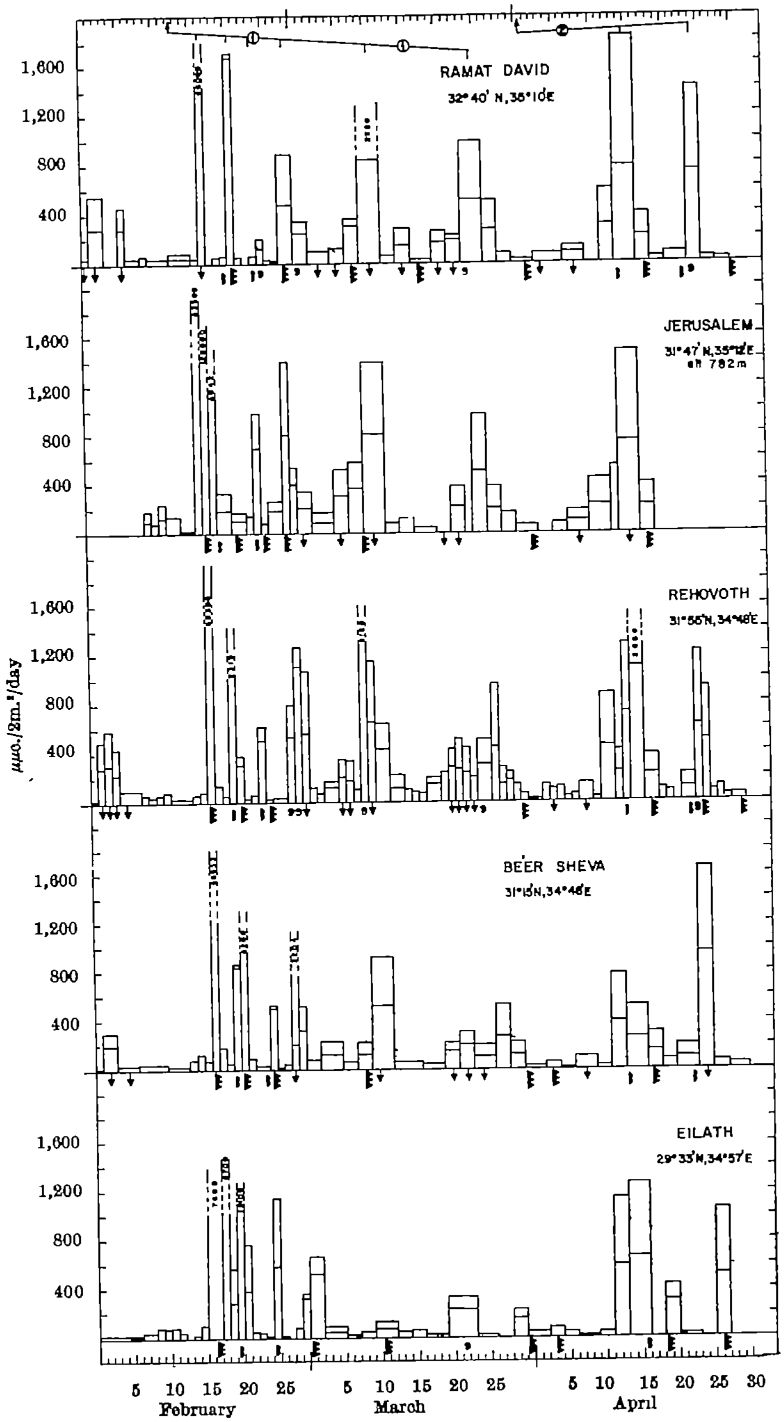

Fig. 1. Deposited fall-out activity at five 'gummed tape' collection stations in Israel, February–April 1960. (Activity on fourth day after collection)

Meteorological symbols : ▮, warm front ; ▮, cold front ; ↓ ; ●, drizzle

The origin date of the activity peaks, as determined by decay measurements, is indicated on the top of the diagram

The passage of a marked cold front accompanied by rainfall on February 28 resulted in an appreciable deposition of activity throughout Israel. This front also trailed back and diffused in the south of the country. Backtracking in the 500-mb. layer shows that air in this layer had passed over the Sahara some days before. Since there is apparently no alternative contamination source, we postulate that this air had picked up activity by vertical mixing from the near surface air while passing over the

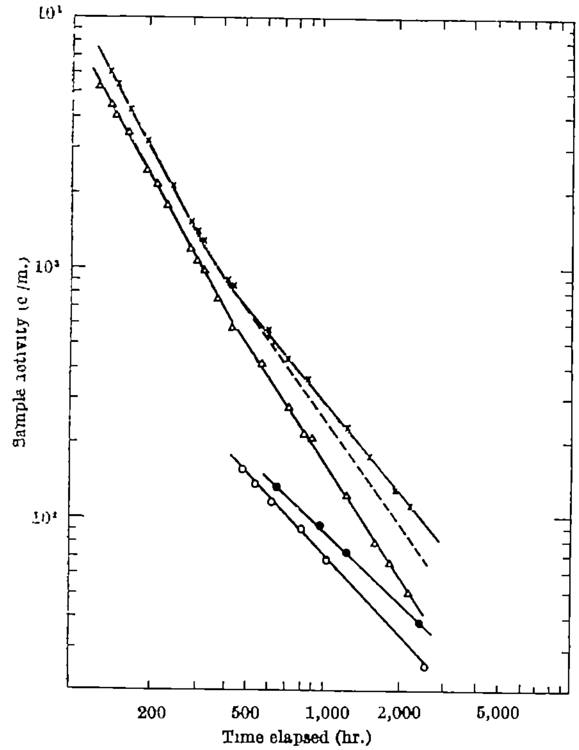

Fig. 2. The decay of representative samples of fall-out activity. △ Activity deposited on 'gummed tape collector' at Rehovoth, on February 17, 1960 (time origin February 13), ×, activity collected by air filtration at Rehovoth on February 17, 1960 (time origin February 13); the broken line shows the decay of the sample corrected for the presence of an average amount of long-lived background activity; ○, activity deposited on 'gummed tape collector' at Rehovoth on April 15, 1960 (time origin April 1); ●, activity deposited on 'gummed tape collector' at Tirat-Yael on April 24–25, 1960 (time origin April 1)

Sahara. It is worth while to note that this assumed mode of transport resembles the mechanism whereby activity from the Windscale release reached Western Europe[3].

During the first week of March good deposition conditions (rain) occurred on a number of occasions, without fall-out being observed. On March 9–10, however, activity was brought down by rainfall, presumably from a nuclear 'cloud' which had circled the Earth with the prevailing westerly winds of the upper troposphere (at an average rate of 65 km./hr.).

During the second half of March unstable cold air caused precipitation throughout the country which was not accompanied by much activity. From March 26 onwards hot desert air moved in from the south, gradually building up sharav conditions. ('Sharav' is a period of hot and dry weather caused either by subsidence owing to the persistence of an anticyclone over the area or by advection of desert air from the Arabian Peninsula or the Syrian Desert.) The presence of this air mass resulted in a substantial increase in the deposition-rate of activity. Peak activities were recorded on March 26 and 27 in the northern part of the country. There was notable deposition even though subsidence conditions prevailed, so that there was no vertical air exchange in turbulent drafts. The influx of cold air during March 30–31 which replaced the hot and contaminated air terminated the heat wave as well as the fall-out.

The deposition pattern of fall-out from the second nuclear test explosion differs in some respect from that described above. No fall-out was detected here during the first ten days following the test in spite of the fact that two cold fronts, accompanied by some rain, passed over this area. This result is in agreement with the fact that the streamlines of upper air (above 3 km.) were due eastward from the test site and therefore probably carried debris on a more southerly route than the one followed after the first test explosion (Fig. 5). The near surface air (up to 1·5 km.) near the test site was, however, seemingly trapped in the desert for some days by a 'high' which extended over Lybia at that time. On April 6 some of this air formed an anticyclonic cell which reached Israel on April 13, its edge advancing as a warm front northward on April 14 and 15 (Fig. 6). The radioactivity measurements (Figs. 1–2) show that this desert air carried with it some activity from the second test. Its slow northward advance is clearly reflected in the results of fall-out measurements of the stations in the south and north. The fall-out came to an end on April 17 on the arrival of cold air from the west. The additional activity peak recorded on April 24 (except at Eilath, where its appearance was delayed) can perhaps be attributed to the passage of a nuclear cloud from the second test explosion which had circled the Earth. This deposition of activity occurred during the passage of a cold front. The decay-rate of the deposited activity is similar to that of activity deposited during the week of April 12–18.

No further significant deposition-rates were recorded up to August 1, 1960.

Air Activity Measurements

The radioactivity of particulate matter in the surface air as determined at Rehovoth Station by air

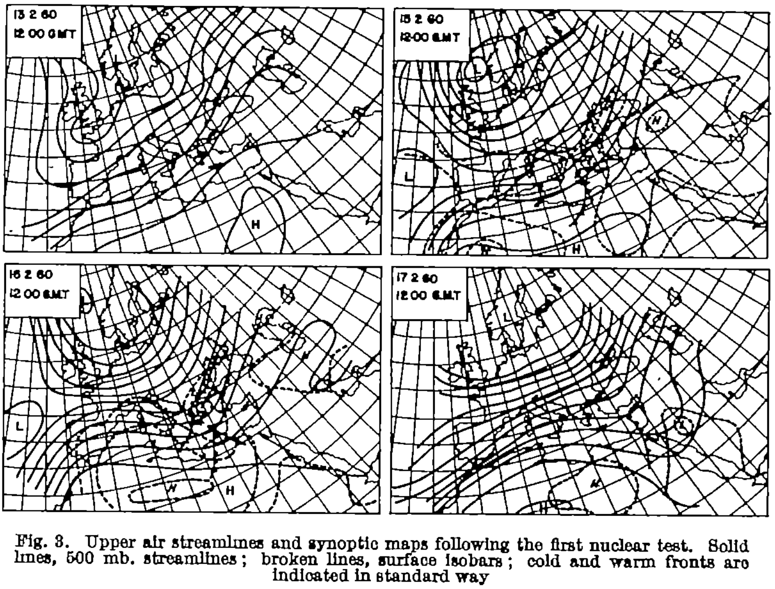

Fig. 3. Upper air streamlines and synoptic maps following the first nuclear test. Solid lines, 500 mb. streamlines; broken lines, surface isobars; cold and warm fronts are indicated in standard way

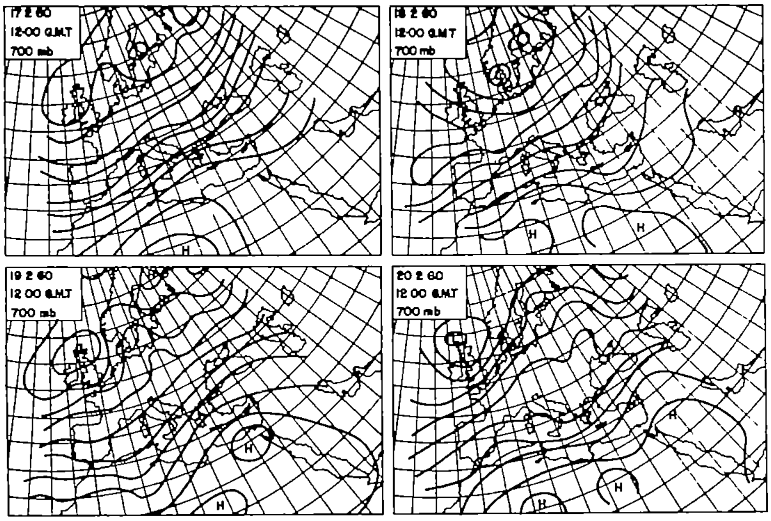

Fig. 4. Streamlines of 700-mb. layer, showing the formation of anticyclonic cells over North Africa and their trajectory into the Levant

filtration with Staplex- and Victoreen-type air pumps, is shown in Fig. 7. It should be noted that the air activity diagram shows less variations than the corresponding 'deposited activity' diagram. There is an appreciable long-lived activity background of about 0·08 μμc./m.^{3}. Marked increases in the air activity were observed only on February 17, March 9, during March 13–14 and March 27–31, on April 14 and 15 and on April 24. Only the peaks of February 17, March 9 and the April activity peaks have their counterpart in the deposited activity diagrams. The decay of the activity collected on these days is very similar to that of the activity deposited on the gummed tape collector, if one takes into account the long-lived background activity (Fig. 2). The decay-rate of the activity collected on the other peak days shows that little activity of recent origin is included in these samples. The relative sizes of the air activity peaks (ratio of signal to average background) recorded on the air monitor after the first nuclear test are smaller by two orders of magnitude (a factor of 30 on the Staplex pumps) than the corresponding peaks of deposited activity, but are about equal for the peaks detected during April following the second nuclear test

Discussion

Deposition of nuclear weapon debris resulting from the Sahara tests was noted on rainless days only when one of two distinct meteorological conditions prevailed: during the passage of cold fronts or during the sojourn of air from anticyclonic cells moving in from the African continent. The deposition pattern was quite different in these cases.

The cold fronts resulted in large amounts of activity being deposited during a short time-interval, the amount apparently depending on the temperature gradient of the front. The observation of activity at ground-level is in this case made possible by the existence of a vertical transport mechanism active to a height of a few kilometres. The coincidence between all measured activity peaks and the existence of such a mechanism shows that no activity can be detected at ground-level unless two conditions are satisfied: (1) The presence of a 'nuclear cloud' aloft; (2) the existence of an atmospheric transport mechanism reaching up to the height of the cloud. It follows that the extent of the nuclear cloud aloft cannot be determined from the duration of the deposited activity peak.

On the other hand, the radioactivity carried by the warm air cells appears in the form of broad peaks on the deposited activity diagrams. Activity was detected even in the absence of any distinct mechanism for vertical air exchange. The duration of these peaks is therefore determined by the time of stay of the contaminated air masses in the area.

Each of the activity peaks recorded during the period under discussion was associated with air which was tracked back directly to the vicinity of the test site, either through the surface or upper-air layers. Meteorological systems which brought air along different tracks during this period contained no measurable amounts of (recent) fission product activity. This simple deposition pattern is probably the result of the tendency of the air-flow to be zonal during this period. The high circulation index indicates the lack of transport of radioactivity on a large scale across latitudes, except for one occasion when a northward protrusion of an upper ridge of high pressure extending from North Africa to Europe may have been responsible for the activity peak reported in the United Kingdom between February 28 and March 1[3]. Judging from our experience with the flow regime observed following the tests, it

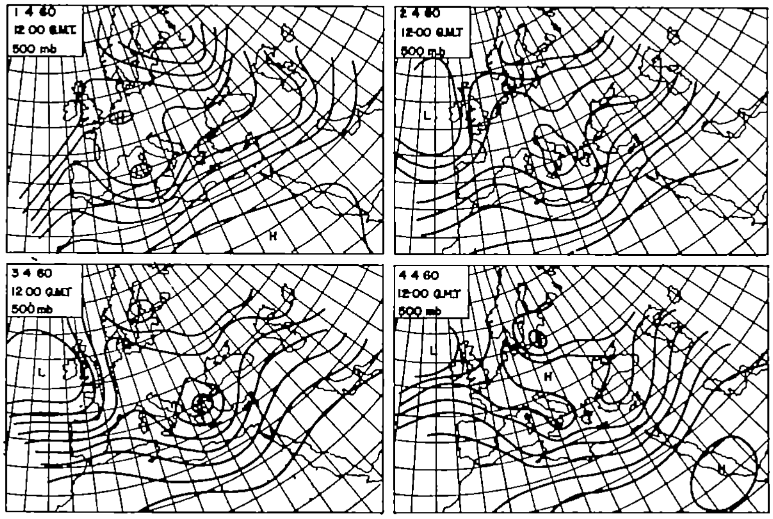

Fig. 5. Upper air streamlines following the second nuclear test

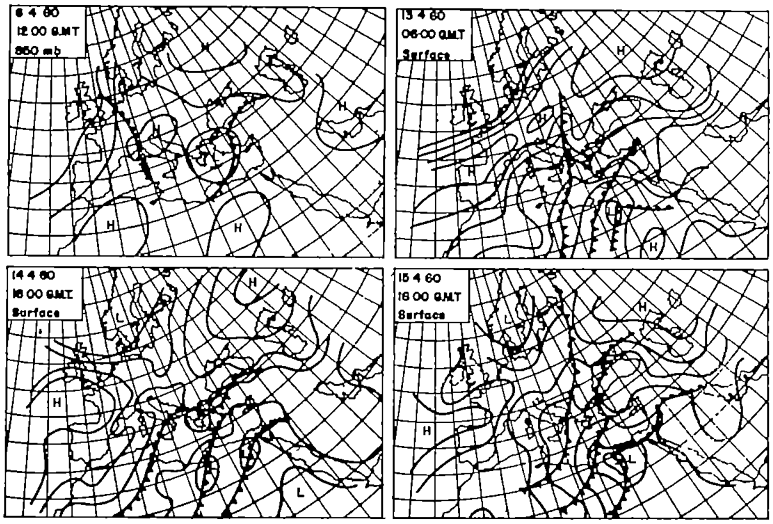

Fig. 6. Map 1: typical synoptic situation which results in activity being confined over North Africa. Maps 2–4: surface pressure distribution (during period April 13–16) showing the development of an east-moving low across the North African coast which caused this activity to move into the Levant

appears that synoptic data are sufficient in a simple meteorological system to forecast quite accurately the transport of radioactivity in the weather layer. This, however, may be very difficult where more complex meteorological systems develop.

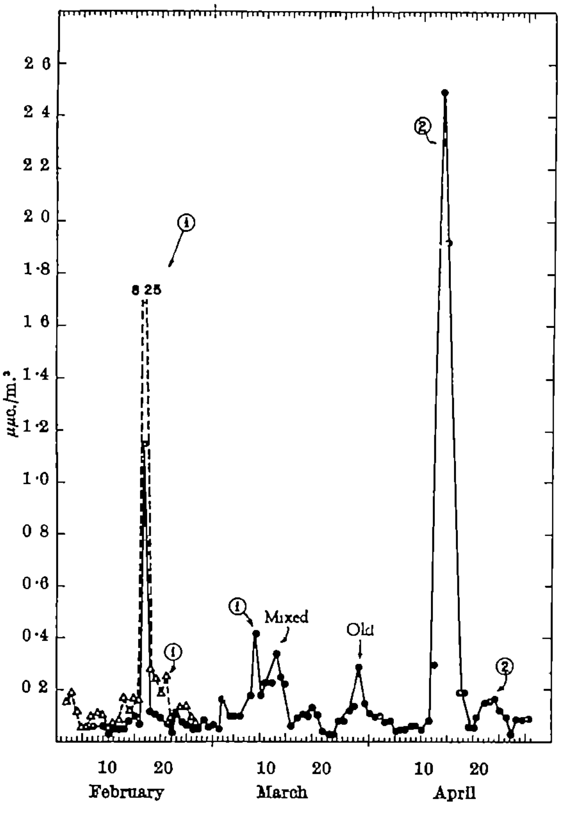

Fig. 7. Activity of particulate matter in surface air at Rehovoth Station, February–April. (Activity is given in units of μμc./m.³ on fourth day after collection.) Δ, Staplex air pumps with *TFA* No. 41 filters; ●, Victoreen, Model 900–2, mobile air monitor. The origin of the activity peaks (first, second or previous nuclear test) is indicated

The surprising result of the measurements is the fact that, for more than a month following each test, every air mass which reached Israel after having been in contact or in exchange with near-ground-level air of the Sahara contained radioactivity. The localized persistence of the radioactivity in the air above the Sahara, inferred from this result, is noteworthy. It is undoubtedly due to the aridity and the semi-stagnant air circulation pattern of the region and hence a peculiarity of the dates and location of the tests. The results of radioactivity measurements at Ghana, reported by Ward and Marr[4], confirm the continuously high air count of the desert air up to the beginning of March.

The debris from the first nuclear test was detected much more efficiently on the gummed tape than by the air pumps. It behaves quite differently in this respect from the material from the second test as well as from the residual airborne activity which is responsible for the appreciable background count on the air-filters but is barely detectable on the gummed tape.

The gummed tape collector is considered (except during showers) to be a sampler of large-sized particles only. The air-filters, on the other hand, are known to be effective in collecting fine particles (larger than $0\cdot01\mu$). The Staplex type air pumps when set up horizontally with these filters are, however, poor collectors of coarser grain particles at the flow-rate used; the continuous air monitor having a curved inlet pipe is even less responsive to the larger particles.

From the different response of the sampling equipment to debris from the two test explosions it is inferred that the debris materials differ considerably in the size distribution of the radioactive particles. The results may be interpreted in terms of (size) spectral shift to larger particles of the atmospheric radioactivity associated with the first nuclear test, relative to the residual atmospheric radioactivity which is responsible for the background counts. This seems to be true for the peak of February 17 as well as for subsequent peaks related to the first test.

Debris from the second nuclear test seemed to be in a more finely divided form. No hot spots were found, and the air monitor proved to be as responsive (signal to background ratio) as the gummed tape collector. The different size distribution of the debris from the two tests is probably related to the fact that the second explosion was an air drop whereas the first test was reported to be a tower experiment.

The results emphasize that the quantitative response of the conventional fall-out detection systems depends on the size distribution of the fall-out material at ground-level as well as on its concentration in the air. A number of discrepancies between air activity and fall-out deposition-rates seen in the literature[5] may find their explanation in the different sensitivity of different monitoring equipment to the size and shape of the aerosol particles. The possibility of detection at ground-level of fall-out material from higher-altitude layers depends, of course, with any monitoring system, on the operation of favourable meteorological transport conditions.

The measurements show the 'gummed tape' to be an efficient collector of fresh nuclear test debris even on rainless days; the activity measured cannot, however, be quantitatively related to true air concentrations of fall-out.

The amount of activity deposited in Israel as a result of the nuclear tests in the Sahara turned out to be insignificant from a public health point of view; it contributed only a small addition to the fission debris accumulated as a result of previous nuclear test explosions.

We are indebted to the Director, Israel Meteorological Service, and to the staff of the Forecasting Division at Lod for the use of synoptic maps and to the Israel Atomic Energy Commission under the auspices of which the measurements were carried out.

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POST-SECONDARY AND HIGHER EDUCATION IN NIGERIA

By Dr. C. T. MORTIMER
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"INVESTMENT in Education" is the title of the report of the Commission on Post-School Certificate and Higher Education in Nigeria*. Sir Eric Ashby was the chairman, and the Commission included American and Nigerian members. It was constituted to investigate Nigeria's needs in education over the next twenty years, and to recommend how these needs might be fulfilled.

The Commission's approach to the problem reflects the optimism which is to be found in Nigeria to-day. It thinks of the country as it will be in twenty years time: a nation of some fifty million people, with industries, oil, and a well-developed agriculture. To provide for this development the Commission's educational proposals are massive, expensive and unconventional. They include doubling the secondary school intake, creating a completely new teacher-training scheme, building six large technical institutes, and increasing the output of graduates from 300 to 2,000 a year; all in the next ten years. This would undoubtedly be beyond the present resources of the Federal and Regional Governments. Only with aid on an international scale can this educational 'crash' programme be accomplished.

A survey of the need for 'high-level' man-power, to enable Nigeria to carry out the programme of economic development, has been made by Prof. Frederick Harbison, of Princeton University. In this context 'high-level' man-power means men and women who have had education beyond school certificate. The minimum need, over the next ten years, is for 80,000 people with some post-secondary education. About 30,000 of these are described as "senior: managerial, professional and administrative", and a majority of them should be graduates. Some 50,000 people will be required with an "intermediate" qualification, calling for two or three years of full-time study after school certificate.

It should be emphasized that these estimates are a minimum which is required to permit the economic growth of Nigeria in the next decade to be equivalent to that in the ten years prior to independence. This has been only 4 per cent per annum in real terms. Moreover, even if this rate of economic growth is sustained, by 1970 Nigeria will still have a lower number of high-level man-power per thousand population than either Ghana or Egypt has at present. Also, during this time Nigeria's population will have increased by about one-sixth of its present number.

The report stresses, however, that in planning educational institutions, it is the rate of flow which is important. The objective is, therefore, to propose an educational machine which will produce at least 8,000 people per annum with some post-secondary education.

In 1958, about 600,000 pupils entered primary schools, which is a substantial proportion of the 800,000 who (at a rough guess) reach the age of seven each year. This represents almost univeral primary schooling in the Eastern and Western Regions, although in the Muslim Northern Region, where the development of Western education has been much slower, only about one-tenth of the young children attend primary schools. In order to fulfil Harbison's estimates, about 5 per cent of those who finish primary school (some 30,000 pupils) should enter secondary schools each year, with a view to slightly more than 1 per cent (8,000 pupils) eventually obtaining a post-secondary qualification. For these objectives to be achieved, four distinct, though inter-related, parts of the educational system must be strengthened or developed. These are: (1) the teacher-training programme; (2) the secondary schools; (3) facilities for technical training; (4) the universities. These topics the Commission considers in detail.

As in all undeveloped countries, much depends on the teacher-training programme. Both an emergency, short-term scheme and also more permanent facilities are recommended. At the base of the educational pyramid the first step is to give an "injection of remedial education" to some 8,000 primary and secondary school teachers, many of whom have only the most meagre of qualifications. This would be in the form of short residential courses of one or two months, held in the universities. The benefits of these courses would be consolidated by a teacher-guidance service of about 1,000 experienced travelling teachers, most of whom would have to be expatriates, at least for some time to come.

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* Investment in Education: The Report of the Commission on Post-School Certificate and Higher Education in Nigeria. Pp v+140. (Lagos: Federal Government Printer; London: Crown Agents for Oversea Governments and Administrations, 1960.) 5*s*. net.

More permanent training facilities will provide for Grade I colleges, offering a qualification which would be a minimum for secondary school teachers, and also Grade II colleges, which the majority of intending primary school teachers will attend. It is hoped that by 1970 the Grade II colleges, which now offer only a two-year post-primary education, will be upgraded and provide training up to school certificate level, together with the teaching certificate, and have an output of 18,000 teachers a year. These colleges will require a large number of graduate and Grade I staff. It is estimated that the output of teachers with the equivalent of Grade I status must be not less than 3,000 a year. The main avenue to this qualification must be two years of formal education after the school certificate, and involves finding 6,000 teacher-training places. The majority of the Grade I teachers will be trained in eight new colleges. The technical institutes will train about 500 teachers a year, who will teach manual subjects, which would become obligatory in all primary and secondary schools. This should help to correct one of the major defects in Nigerian education, namely, a strong bias towards the traditional literary and academic subjects, which is reflected in a lack of respect for manual skills and technical achievement. The training of graduate teachers is considered under the section dealing with the universities.

The secondary schools should be able to take about 30,000 pupils a year. At present there are facilities for only 12,000 entrants annually, so that 18,000 places a year must be created. This is equivalent to some 600 secondary school streams. From the 30,000 who will enter secondary schools, it is expected that 25,000 will obtain a school certificate, and of these 5,000 would proceed to some intermediate training, while 3,000 would continue with sixth-form studies, with the view of entering universities.

The Commission is strongly in favour of sixth-form work being carried out in secondary schools, rather than in special colleges, or in preliminary courses at the universities. At present there are sixth forms in only 22 schools, catering at most for 500 pupils a year, so that over the next decade another 100 sixth-form streams must be created (which, incidentally, will require at least 400 additional highly qualified teachers). But these sixth forms cannot wait until they are built up from the junior classes of new secondary schools. The Commission therefore recommends that national high schools be established, consisting initially only of sixth forms (the pupils being admitted from other schools in the area). In time junior classes would be grafted on. In addition, selected secondary schools should be encouraged to develop sixth forms.

It is estimated that some 3,000 technicians should be trained each year. The most efficient development of technical education would be to have about six large institutes in centres of population. For the next ten years the institutes at Enugu, Ibadan, Kaduna, Yaba, together with that planned for Benin City, that contemplated at Port Harcourt, and, it is hoped, one at Kano should suffice.

Part of this scheme is 'self-generating' and should produce, by 1970, enough men and women to staff the 18,000 Grade I appointments in primary and secondary schools, in teacher-training colleges, in technical institutes, and in the supervisory teaching scheme. It will provide entrants to the universities, some of whom will return to fill the 7,000 graduate teacher appointments. But unless some initial momentum is given the whole machinery will get under way too slowly. To "prime this educational pump" the Commission hopes it will be possible to import a corps of teachers into Nigeria on short-term contracts. Graduates would have to be engaged at the rate of 1,000 a year between 1962 and 1966. At the same time a group of Nigerians (some 600 a year) would be sent overseas for university training to prepare for teaching. This measure would produce enough Nigerians to replace the short-term, expatriate teachers by 1970. During this period it is hoped that the output of graduate teachers from Nigerian universities would rise to about 800 per year.

For the continued growth of Nigeria's economy at its present modest rate it is estimated that the country needs at least 2,000 graduates a year. The student population in Nigerian universities at present is about 1,500, and the output of graduates is 300 a year. Also, about 500 Nigerian graduates return each year from overseas universities. The Commission recommends that at least 7,500 student-places should be available in Nigerian universities by 1970. Three aspects of this proposed university expansion are considered in detail: the range of studies, the sites for new universities, their financing and government.

There must be more diversity and flexibility in university education than is found in the British system if it is to meet the needs of the Nigerian people. The British system of university education suits Britain because there are many alternative routes to professional training. These are lacking in Nigeria. However, greater diversity must not carry with it lowered standards. African studies, commerce, teaching, engineering, medicine, agriculture, law and extra-mural studies are fields of study which are vitally important.

The most obvious need for innovation in Nigerian universities is in African studies. It must be a first duty to foster study of African history, languages, and societies, and when a sufficient body of knowledge has been built up these could be incorporated into undergraduate courses.

In Britain, most accountants, bankers, company secretaries, insurance managers and transport executives are trained 'on the job'. In America, professionals in these fields are college-trained and have degrees. An amalgamation of these two methods would suit Nigeria's needs: to offer degree courses in commerce and business administration, preferably to be taken as sandwich courses, where the student would alternate some months of office work with some months of study.

Nigeria will need 800 graduate teachers a year. Only a minority of these teachers will require a concentrated training in a narrow field, for sixth-form teaching. For the great majority, a degree based on a general curriculum would be more useful. The Commission proposes that all Nigerian universities should institute a degree of B.A. (Education). The course would take three years, and would consist of four subjects in the first year, three in the second, and three in the third year. Teaching practice and a course in pedagogy would also be included.

For many years to come Nigerian specialists in engineering will seek postgraduate experience overseas, but for her indigenous engineering training the country needs degree courses with an emphasis on the practical side, and not over-sophisticated.

The needs of Nigeria for medical services are totally different from those of Britain. It is unreasonable to assume that the standards of attainment for Nigerians practising in Nigeria must be the same as those of Englishmen practising in England. Also, in Nigeria to-day there is one doctor to every 40,000 people (as compared with a ratio of 1 : 1,000 in Britain) so that there is a tremendous need to increase the output of doctors. Specifically, the Commission makes three main recommendations ; (*a*) doubling the output of the University College Hospital, Ibadan ; (*b*) instituting a locally recognized examination, for those who fail to qualify for the medical degree at Ibadan ; (*c*) re-orientating the medical courses to emphasize public health, preventive medicine and pædiatrics.

The Commission was favourably impressed with the state of agricultural (degree) courses at the University College, Ibadan ; but the flow of graduates (20 a year) is pitifully inadequate to meet the nation's need of 200 a year.

So far as the siting of new universities is concerned, the Commission proposes that there should be four large universities, one in each of the three Regions and one in Lagos, the federal capital, rather than a number of smaller universities. These universities should be independent of one another ; they should be national institutions with all three Regions equally represented on their governing bodies ; and they should be financed largely from federal funds on the advice of a national universities commission.

It is recommended that the three regional branches of the Nigerian College of Arts, Science and Technology should become centres of university education. The Ibadan branch would be joined to the adjacent University College, Ibadan. The branch at Enugu, in the Eastern Region, would become part of the new University of Nigeria, at Nsukka. It would be used to develop departments of engineering, metallurgy, surveying and the social sciences, and also be the headquarters of an institute of education. The Zaria branch of the Nigerian College would become the centre of a university in the Northern Region, which would also embrace the Ahmadu Bello College of Arabic Studies, at Kano, and the Veterinary Research Institute at Vom. A particular proposal about this new university in the Northern Region is that opportunity should be given for undergraduates to read combinations of subjects drawn from different disciplines.

The University at Lagos would be an unusual type of institution, which would be non-residential, and would have three main departments. These would be a School of Commerce and Business Administration, offering both day and evening courses, a School of Economics and Social Studies, and a Department for Correspondence Courses. Although this would offer only a limited range of courses, it is believed that it would encourage large numbers of men and women, who would otherwise not have the opportunity, to improve their education and fit them for more responsible posts.

This is an outline of the Commission's main proposals. What it recommends is a stupendous undertaking, and will cost large sums of money. The Nigerian people will have to forgo other things they want, so that every available penny is invested in education. Even this will not be enough. In the words of the report, "Nigerian education must for a time become an international enterprise".

VERTEBRATE LOCOMOTION

A SYMPOSIUM on "Vertebrate Locomotion", the fifth in the series sponsored by the Zoological Society of London, was held at the rooms of the Society on Wednesday, November 9, 1960.

Sir James Gray opened the meeting with a paper on the general principles involved in studying the mechanics of vertebrate movement. He stressed the difficulty of providing an exact treatment of the problems of aquatic and aerial locomotion and gave examples from his own work and that of his students on these and on the more amenable problems of tetrapod movement. Here, exact measurement of forces exerted by the limbs on the substratum provided a basis for analysing the locomotor system ; from these results it was demonstrated that the muscular system must be treated as a single integrated whole.

Dr. R. Bainbridge, after enumerating and commenting on the many still unsolved problems in fish locomotion, concentrated on one of these—the relationship of power output, in terms of known data for muscle, to drag, calculated on various simple assumptions.

He showed that within the wide range of experimentally established estimates for the power output of different vertebrate and invertebrate muscles, it was possible to account for the observed steady speeds of progression of most small fish as well as of whales and dolphins without the need to assume completely laminar flow over the body surface. A surprising exception was provided by the barracuda.

Dr. G. M. Jarman contributed a brief note on the form of intermuscular bones and myocommata in the fish myotome. He suggested that the conical surface of a myocomma is inclined at such an angle to the longitudinal (contractile) axis of the muscle that the connective tissue mass does not change in area or thickness and therefore waste the contractile effort of the muscle.

Mr. E. H. Watts gave an account of some experiments in designing ships on lines which imitated certain features in fast-swimming fish. Perhaps the most significant of these practical exercises was the provision of lateral keels on either side of the stern, similar to those found in scombroid fishes and certain sharks. These structures slightly increased the propulsive efficiency and greatly reduced the minimum turning circle of the vessel. A more surprising result, which did not appear explicable on the models of fish locomotion described earlier by Dr. Bainbridge, was that the artificial creation of turbulent flow along the hull form appeared in some ships actually to increase the propulsive efficiency by reducing the drag.

The considerable variation in form and flexibility of the sacro-iliac joint in the anuran amphibian was described by Dr. H. P. Whiting. In *Xenopus* and

Discoglossus respectively, longitudinal sliding and horizontal rotation of the pelvis on the vertebral column are very marked; *Rana* shows the more commonly known rotation in a vertical plane. The different motions are consistent with observed differences in body movement of the normal animals, and suggest that an important shock-absorbing property, as well as a possible locomotory contribution, is provided by the musculature of the sacro-iliac joint.

Dr. K. M. Backhouse described the fish-like lateral motion of the hind feet of the seal when swimming in water; and contrasted this with the movement on land, where progression is achieved by dorso-ventral flexion of the trunk coupled with a hitching action of the fore limbs. Slow-motion films illustrated the terrestrial locomotion, in which the highly flexible metacarpophalangeal and interphalangeal joints of the fore limb play an important part.

Prof. E. J. Slijper, of Amsterdam, gave an account, also illustrated by film, of locomotion and locomotory organs in whales and dolphins. An interesting point to which he directed attention was the fact that cruising speed and maximum speed of the small dolphins was about the same as that for the much larger whales. He suggested that this could be explained partly by the better mechanical organization of musculature in dolphins, but also on the assumption that the flow of water over the body surface of the dolphin was almost wholly laminar, while it was partly turbulent in whales.

The power of the flying bird was discussed by Dr. R. H. J. Brown. He surveyed the various attempts to estimate this from the earliest date of Lilienthal to the more recent figures based on wind-tunnel tests, oxygen consumption figures and gliding parameters of birds in actual flight. These data suggest that the aerodynamic efficiency and the muscle-power must be very high even in normal sustained flight; greatly increased power must be available for take-off and climbing.

Terrestrial locomotion of dogs and horses was dealt with by Prof. C. W. Ottaway, who described the highly co-ordinated and integrated action of limb and shoulder muscles in the normal walking stride, and illustrated the value of nerve section and tenotomy as a method of studying the role of individual muscles.

Finally, a contribution on arboreal mammals by Dr. J. R. Napier reviewed the evolution of prehensility in the primate hand. The inward turning of the thumb which characterized true opposability required a deep carpal tunnel brought about by the set of trapezium and scaphoid, together with the existence of a saddle joint at the carpo-metacarpal articulation. True opposability is only attained in Old World monkeys, and was evolved fairly late in primate history.

The symposium was well attended, and the discussion which followed each session, though necessarily brief, raised a number of interesting points made from personal observation on locomotory behaviour as well as on the theoretical principles involved. It was fittingly concluded by the showing of a film, still in its partially edited condition, from the Zoological Society's television unit, demonstrating various modes of running and jumping by man and by animals from the collection. The slow-motion sequences collected together in the film, which was introduced by Dr. Fay Hall, were particularly interesting and instructive. John E. Harris

OBITUARIES

Prof. W. J. Duncan, C.B.E., F.R.S.

William Jolly Duncan, Mechan professor of aeronautics and fluid mechanics in the University of Glasgow, died on December 9 at the age of sixty-six. He had a very varied experience. Born on April 26, 1894, he was the son of Robert Duncan, M.P., senior partner of Ross and Duncan, engineers, of Glasgow. He was educated at Dulwich College and the University of London. He served in both World Wars, during the First in France and Flanders and later at the Aircraft Inspection Directorate. In the Second World War he worked for a time at the Royal Aircraft Establishment, and then as head of the Air Defence Research Establishment at Exeter. In 1945 he was sent to the Luftfahrtforschungsanstalt Hermann Göring, at Völkenrode, to supervise the disposal of German aeronautical equipment. He married in 1936 the daughter of G. S. Baker, who was then superintendent of the William Froude Ship Tank at the National Physical Laboratory, and had a family of four—all girls.

Duncan was appointed to the scientific staff of the Aerodynamics Division, National Physical Laboratory in 1926, and it was there that I, superintendent of the Division, first came to know him. In his work there, he was closely associated with the late Dr. R. A. Frazer, and A. R. Collar, now professor of aeronautical engineering in the University of Bristol. This team of three scientists was entrusted with the investigation of the new problem of aircraft flutter, a problem they were able to solve fairly completely, and in particular to lay down design rules whereby flutter could be avoided. In the process they greatly developed the theory of matrices, and together wrote a book, "Elementary Matrices", published in 1938, which has been reprinted four times and is a standard work on the subject. In 1934 Duncan left the National Physical Laboratory to become the first head of the Department of Aeronautics at Hull, and in 1938 he was made the first Wakefield professor there.

At the end of the War a group of four was entrusted with the search for a suitable site for the proposed new College of Aeronautics; Duncan and I were two of these, and we both became very interested in this revolutionary new idea in postgraduate training. When the College was eventually established at Cranfield, I became its first principal and Duncan was appointed professor of aerodynamics, and became also deputy principal three years later. I can say with certainty that the major part in the formulation of the teaching methods to be adopted in the new College was due to Duncan. It was during this period that he and I made arrangements with the Cambridge University Press for a series of aeronautical books, and Duncan's work on "Control and Stability of Aircraft" was the first of this series. Incidentally, he also wrote the first report of the College of Aeronautics to be issued.

Duncan's teaching at the College was outstanding in excellence, as is witnessed by the fact that many of his students who specialized in aerodynamics now occupy major positions in the aircraft industry and in aeronautical research. He left Cranfield in 1950 to become the first Mechan professor of aeronautics and fluid mechanics in the University of Glasgow, a post which he held until the end of his life. He had been chairman of the Aeronautical Research Council since 1957, and was very active not only on the Council but also on many of its committees and sub-committees.

Prof. Duncan was an able mathematician and a first-class engineer, painstaking and thorough in all his work, and a great inspiration to those who had the good fortune to work with him. He was elected a Fellow of the Royal Society in 1947 and was appointed C.B.E. in 1953. His publications, besides the two books already mentioned, include one on "Physical Similarity and Dimensional Analysis", together with numerous reports and papers published in the Reports and Memoranda of the Aeronautical Research Council, the *Proceedings of the Royal Society*, and the *Philosophical Magazine*. His death will be felt as a great loss by his many friends and collaborators, and by the world of mechanical engineering in general.

ERNEST F. RELF

Prof. J. G. Andersson

PROF. JOHAN GUNNAR ANDERSSON, formerly director of the East-Asiatic Collections at Stockholm, was one of the Swedes who have made lasting contributions to our knowledge about the natural resources of China and the history of the civilization of that country.

He was born on July 3, 1874, at Knista in the province of Orebro, and died in Stockholm on October 29. After studying at the University of Uppsala, he obtained there the degree of M.Sc. in 1901, became D.Sc. in 1902, and was appointed professor of geography in 1906. Already during his years as a student he devoted himself to the examination of the Silurian deposits of his native county of Närke and of Öland. These investigations resulted in a couple of palæontological communications and a study of the distribution of phosphorites in the Silurian.

As a young student he took part in several expeditions into the Arctic regions. Thus he was a member of the expedition to Spitsbergen led in 1898 by the Swedish polar explorer A. G. Nathorst. In 1899 Andersson undertook an expedition of his own to Beeren Island. The geological results of this undertaking he described in his doctoral thesis, "Über die Stratigraphie und Tektonik der Bären Insel". When in the years 1901–3 Otto Nordenskjöld undertook his famous Antarctic expedition, Andersson was second in command, and when Nordenskjold together with some other members had established winter quarters on Snow Hill, Andersson took over the leadership of the expedition to South Georgia, the Falkland Islands, and Tierra del Fuego, and also during the second trip of the vessel to the Antarctic. Afterwards Andersson left the vessel with two companions in order to reach Nordenskjold's winter quarters by land. Before reaching it he was, however, forced to spend a winter under very trying conditions. He then joined Nordenskjöld's group in the following spring. In the meantime, their ship foundered in the pack-ice, yet without loss of human lives. This expedition and its rescue by an expedition sent out from Argentina forms one of the more thrilling chapters in the history of the exploration of the polar regions.

In 1906 Andersson was made head of the Geological Survey of Sweden. After having had leave of absence since 1914, he left this post in 1916. During his comparatively short time as head of this organization, he introduced a number of new and important investigations into its programme. This applies first of all to the production of oil and radium from the oil-shales. The organization of the Geological Survey as established in 1914, which has remained in force to the present day, is to a large extent due to him. During the Eleventh International Geological Congress, held at Stockholm in 1910, he was, partly in collaboration with H. Munthe and A. Gavelin, the editor of the publications of the Congress.

As mentioned already, Andersson had obtained leave of absence in 1914 from his post as head of the Geological Survey, and had entered the service of the Chinese Government as mining adviser and organizer of the geological exploration of the ore and mineral resources of China. It was during this period that he became widely known as an explorer of China. In addition to the study of ore geology, he found time for extensive studies of the fossil faunas and floras of China as well as for remarkable archæological excavations that threw light on the earliest settlement of China. In his endeavours he enjoyed powerful support from the Chinese Government and from the Swedish Parliament. From this field of activity 'China Gunnar', as he was called in many circles, brought back extensive collections which are now in different Swedish museums.

After his return from China, Andersson was appointed in 1926 to a chair of geology in the University of Stockholm. From this he retired, however, in the following year, in order to devote himself to the arrangement of his collections, and to a new trip to China. On his return from his second visit to China he was made director of the East-Asiatic Collections in Stockholm, and was given a personal chair in East-Asiatic archæology which he then occupied until 1939.

Andersson published the results of his researches and explorations in a great number of scientific papers. His studies in the Far East were summarized in his "Topografisk-arkeologiska studier i Fjärran Ostern" (1939). He had a great gift of popularizing his knowledge, both as a lecturer and as an author. In 1926 he published his account of China, "Draken och de främmande djavlarna", followed by "Den gula jordens barn" (1932), the autobiographical "Kineser och pingviner" (1933), and "Under brinnande krig" (1938). He surveyed his own life and his life's philosophy in a book, "Mitt liv och min tro" (1956).

ERIK ÅHMAN

Dr. Louis Clarke

LOUIS CLARKE'S death on December 13 leaves a gap in the hearts of his innumerable friends, for he was a much-loved man. He was wealthy and was blessed with intelligence and common sense, with a lively understanding of human nature and with the standards of behaviour of his Christian Victorian upbringing. He travelled widely after leaving Cambridge, especially in South America and

Abyssinia, and was extremely knowledgeable in matters of archæology and art. Hence his two official jobs, first as curator of the Museum of Archæology and Ethnology at Cambridge, and later as director of the Fitzwilliam Museum.

But the outstanding fact was that Louis could neither be described as an academic nor as an ordinary person. He took a very modest degree, and, though in later life his eminence was such that his University gave him an honorary doctorate, he was essentially an example of the fine fruit of what England so specially has produced in the past: he was a distinguished amateur. This is high praise, and it was only possible because in his early life his intellectual energies had not been canalized into a narrow channel: he had had opportunity and leisure to cultivate wide interests and friendships and to gain a broad, wise outlook on life. His knowledge became encyclopædic: he could give you the provenance of any object you might offer for his inspection! Those who knew him well will never forget his enormous, cheery voice resounding through the two Museums he successively controlled, nor his capacity, possibly unrecognized by himself, to unite any team with which he worked into a whole.

At the Museum of Archæology and Ethnology, where he followed Baron von Hügel and Dr. A. C. Haddon and where much had been done to build up a new Museum, it was nevertheless under his direction that it became what it is to-day. He was extremely generous, too, in his gifts to the collections, and much more generous than people knew throughout his life to young or struggling archæologists, financing 'digs' in out-of-the-way parts of the world and adding the results of their excavations to the Museum collections. At the Fitzwilliam his influence was at first to some extent crippled by the War and all that world upheaval meant to a great art museum, but afterwards he was able to do much to further the objects of that universally known institution.

But facts give no picture of the man, with his kindly humour and tremendous knowledge of people, a knowledge which began in his own family of thirteen brothers and sisters and extended to include savants from all over the world. Above all, there was nothing ungenerous in his whole make up, and he has been an ornament to the Cambridge of his generation. For some years his heart had given him trouble, and he died quietly in Cambridge in his eightieth year on December 13.

M. C. BURKITT

NEWS and VIEWS

Geological Society of London: Awards

THE Council of the Geological Society of London has made the following awards for 1961: *Wollaston Medal*, to Prof. Roman Kozlowski, emeritus professor of palæontology, University of Warsaw, in recognition of his distinguished contributions to palæozoology, especially to knowledge of the graptolites; *Murchison Medal*, to Mr. W. N. Edwards, assistant director, Geological Survey of Great Britain (Leeds), for his contributions to the stratigraphy and structure of the Coal Measures, particularly in the Yorkshire–Nottinghamshire Coalfield; *Lyell Medal*, to Dr. J. V. Harrison, lately reader in structural geology, University of Oxford, for his contributions to structural geology and his pioneer work in remote countries; *Bigsby Medal*, to Prof. Alwyn Williams, professor of geology, Queen's University, Belfast, in recognition of his work on the Lower Palæozoic stratigraphy, palæontology and structural geology; *Wollaston Fund*, to Dr. E. A. Vincent, reader in mineralogy, University of Oxford, for his work on the iron–titanium minerals and on geochemistry; *Murchison Fund*, to Dr. J. Ineson, district geologist (Water Department), Geological Survey and Museum, London, for his fundamental contributions to the investigation of ground-water resources in Britain; a moiety of the *Lyell Fund* to Dr. R. G. West, demonstrator, Department of Botany, University of Cambridge, for his contributions to the geology and palæobotany of the Pleistocene of the British Isles; another moiety of the *Lyell Fund* to Dr. W. T. Dean, Department of Palæontology, British Museum (Natural History), London, for his work on the stratigraphy and palæontology of the Middle and Upper Ordovician in Great Britain.

Royal Society and Nuffield Foundation Commonwealth Bursaries

AWARDS under the Royal Society and Nuffield Foundation Commonwealth Bursaries Scheme have been made as follows: Mr. J. Attridge, lecturer in zoology, Birkbeck College, to assist him in doing further work on the Dinosaur beds, and other fossil reptile deposits, of the Karroo System in Southern Rhodesia, from June to October 1961; Dr. M. M. Chakrabarty, reader in applied chemistry, University of Calcutta, to assist him in studying new techniques for the investigation of fatty oils at the Prairie Regional Laboratory, Saskatoon (and possibly also at the Paint Research Station, Teddington), for about six months from March 1961; Prof. J. H. O. Day, professor of zoology, University of Cape Town, to enable him, for about nine months from February 1961, to study type specimens at the British Museum (Natural History) in connexion with the preparation of a monograph on the South African Polychaeta; Dr. W. H. Elliott, Biochemistry Department, John Curtin School of Medical Research, Australian National University, Canberra, to assist him in visiting Cambridge for about a year from September 1961 in order to study microbiological techniques; Dr. D. E. Hoare, lecturer in physical chemistry, Queen's College (Dundee), University of St. Andrews, to enable him to study reactions of hydroxyl radicals with hydrocarbons at the National Research Council, Ottawa, from April to September 1961; Mr. C. R. Horrell, of the Department of Agriculture, Uganda, to enable him to visit Queensland from January to April 1961 to study the development of tropical pasture grasses and legumes for the production of temporary leys; Dr. C. A. Hurst, senior lecturer in mathematical physics, University of Adelaide, to assist him in visiting Edinburgh for about eight months from February 1961 in order to investigate the properties of relativistic two-body equations; Prof. J. C. Jaeger, professor of geophysics, Australian National University, Canberra, to enable him to carry out a study of stresses in deep mines and correlation with seismic observations, at the Bernard Price Institute, Johannesburg, for about three months from November 1961; Mr. I. C. McKellar, of the New Zealand Geological Survey, to assist him in studying glacial geology, Pleistocene stratigraphy and techniques in glaciology in British Columbia, during April and May 1961; Dr. S. S. Qadri, reader in zoology, University College of Science, Osmania University, to enable him to learn protozoological techniques at the London School of Hygiene and Tropical Medicine for a year from January 1961.

Electrical Research Association: New Appointments: Dr. R. H. Golde

FOLLOWING the retirement of Mr. H. M. Lacey on September 30, Dr. R. H. Golde was appointed head of the Surges and Transformer Department of the Electrical Research Association. Dr. Golde is secretary of the Study Committee on Lightning and Surges of the Conférence Internationale des grands réseaux électriques, a member of the International Committee on Lightning Protection and of several British Standards Institution, Institution of Electrical Engineers and International Electrotechnical Commission committees. He was educated at the Technical University of Berlin. He joined the Electrical Research Association in 1935 and took his Ph.D. degree at Queen Mary College, London, and has been awarded the D.Sc. (Eng.) by the same University.

Mr. M. Waters

MR. M. WATERS has been appointed deputy head of the Surges and Transformer Department. Mr. Waters received his early training with A. Reyrolle and Co., Ltd., and during his apprenticeship there he won the scholarship of the North East Coast Institution of Engineers and Shipbuilders, which enabled him to proceed to King's College, Newcastle upon Tyne, where he graduated with honours in electrical engineering. Mr. Waters returned to Reyrolle's, working in the Research Department. After gaining experience in power station construction with Parolle Electrical Plant Co., Ltd., he joined the Electrical Research Association in 1946 to undertake research on power transformers.

Mr. L. Gosland has been appointed deputy director of the Electrical Research Association. This appointment is additional to his position of research manager, which he will continue to hold. Mr. C. G. Garton and Mr. E. W. Golding are appointed assistant directors of the Association. Mr. Garton will continue as head of the Materials Department, and Mr. Golding will continue as head of the Rural Electrification Department, and also as overseas liaison officer.

The British Association for the Advancement of Science: Norwich Meeting

THE annual meeting of the British Association for the Advancement of Science for 1961 is to be held at Norwich during August 30–September 6. This will be the third time that the meeting has been held at Norwich, the previous occasions being during 1868 and 1935, and again it promises a programme with a strong East Anglian interest. Concurrently with the main meeting will be a series of four illustrated lectures for young people, who will themselves organize an exhibition designed to illustrate the scientific work being carried out in the Norwich and county schools. Appointments to the three special lectures are as follows: Kelvin Lecture, Prof. D. J. E. Ingram, professor of physics, University College of North Staffordshire, on investigations in the microwave region of the electromagnetic spectrum; Darwin Lecture, Dr. Judith Milledge, lecturer in crystallography, University College, London, possibly on the importance of borderline subjects—with special reference to crystallography; Lister Lecture, Dr. R. G. Lipsey, reader in economics, University of London, on a subject connected with theory and observation in economics. In addition to the programme for young people at Norwich, Junior British Association meetings will be arranged in 1961 in the West Midlands, Cornwall, Sheffield and Tees-side. The first of a new venture—"Science Fairs"—will be organized in Manchester during March (in collaboration with the Manchester Federation of Scientific Societies), in Tees-side during December and at Newcastle upon Tyne at Easter 1962. Further information concerning the activities of the Association can be obtained from the British Association for the Advancement of Science, 18 Adam Street, Adelphi, London, W.C.2.

Theology and the Sciences at Birmingham

THE University of Birmingham is undertaking a development in its Department of Theology of more than ordinary interest. A second chair, provided by the Edward Cadbury Trust, has been established for a period of seven years in the first instance. This chair will be filled by someone whose interests stretch across the borders between the natural sciences, philosophy and theology. It is hoped that the new professor will pioneer research and teaching in this general area. In Birmingham the sciences have always been strongly represented, and, at present, half the students in the University are reading for degrees in the Faculty of Science. Moreover, last October the Faculties of Arts and Law moved to Edgbaston, and, for the first time for nearly sixty years, all the departments of the University were gathered in a single place. This development seemed to offer an opportunity for interesting and profitable work in exploring the little-known territories where theology, the natural sciences and philosophy meet. The Department of Theology itself, now twenty years old and growing steadily, is non-denominational and widely representative.

Statistical Review of England and Wales for 1958

THE Registrar General for England and Wales has recently issued the third part of his "Statistical Review for 1958", containing the commentary on the vital events of that year (Pp. xiv + 222. London: H.M. Stationery Office, 1960. 13*s.* net). In this volume, which appears after the medical and civil tables, the official statisticians take a careful look at the figures and discuss the implications and trends of vital rates. During 1958, the home population rose by 199,000, of which 185,000 was natural increase (excess of births over deaths), and 14,000 the balance of migration, which it must be remembered includes migration from Scotland and Ireland. It is estimated that there was a net loss of 5,000 on overseas migration, and a net gain of 19,000 on migration within the United Kingdom. Marriage-rates continued to be high, and a continuation of 1958 nuptiality would imply that only 5 per cent of the population would have remained single at age 50, a lower proportion than any ever actually recorded. The number of unmarried men aged 17½–45 exceeded that of unmarried women aged 15–42½ by 6 per cent; only seven years earlier in 1951 this figure had shown a 3 per cent deficiency. On the reverse side, new divorce petitions continued to decline in number;

the 26,000 petitions filed in 1958 were the lowest number recorded since the end of the Second World War. The number of live births was 741,000, the highest figure since 1948. One-eighth of these births were conceived outside marriage, but 60 per cent of these were legitimized by the parents marrying before the birth of the child. It was still, however, premature to say whether this number indicated an increase in total family size, or merely a temporary change in the timing of births within marriage, though it was clear that the rates implied that the population was replacing itself with a margin to spare. In the extensive analysis of mortality, there is a comment on the gap between male and female death-rates. The male rate is now more than double the female in two age groups: 15–24, where men have much higher death-rates from accidents, and at ages 55–64, where the principal contributions are made by coronary disease, with a male rate 3·6 times that of the female, cancer of the lung (9·7) and bronchitis (5·2). There is the usual extensive discussion of cause of mortality. The volume contains a special note on deaths following vaccination and other prophylactic inoculations, and on deaths following from therapeutic misadventure.

Euratom Translation Office at Brussels

THE European Atomic Energy Community (Euratom), the United Kingdom Atomic Energy Authority and the United States Atomic Energy Commission have agreed to pool their efforts to collect and disseminate information concerning translations of nuclear literature, especially from such languages unfamiliar to Western readers as Russian and Japanese. A central information office, called 'Transatom', has therefore been established at Euratom's Brussels headquarters. The office publishes a monthly *Transatom Bulletin*, which lists existing translations recently reported to the Brussels office as well as new translations planned by international or national institutions and private firms in the European Community, the United States, the United Kingdom and in other countries. All data relating to translations, including those made before the establishment of Transatom, are being collected and recorded in a master file system at Brussels. Copies of this card file have been offered to appropriate institutions in countries with great interest in the nuclear field. Contacts have been established in order to avoid duplication of work when the European translation centre, to be established at Delft, Holland, is set up. The scope of this institution is much wider: it will cover all scientific and technical material in the field of exact sciences. The *Bulletin* is available on a subscription basis from Transatom, 51 Rue Belliard, Brussels, at 8 dollars per annum, or 16 dollars air mail.

Use of Film in Scientific Research

THE Department of Scientific and Industrial Research has set up a working party to consider national needs in the field of scientific film. It is especially interested in the aspects of film as a research tool and in communicating research results. To assist the working party the Department, in co-operation with the other research councils, the Atomic Energy Authority and some Government Departments, is circulating a questionnaire to industry, universities and research organizations. The working party consists of: Dr. W. L. Francis (chairman); Mr. Edgar Anstey, British Transport Commission, president of the Scientific Film Association; Mr. D. E. H. Densham, Ministry of Aviation: Sir Arthur Elton, Associated Electrical Industries; Mr. C. Engel, Medical Illustration Department, Guy's Hospital; Prof. G. E. H. Foxon, Guy's Hospital, president, British Universities Film Council; Mr. L. E. Hallett, Royal Photographic Society of Great Britain; Prof. C. H. Waddington, Institute of Animal Genetics, Edinburgh. Three members of the Department of Scientific and Industrial Research are also included: Mr. H. E. Beckett; Mr. L. Poole: and Mr. R. E. Overbury (secretary). Further information can be obtained from the Information Division, Department of Scientific and Industrial Research, 14–18 Cornwall Terrace, London, N.W.1.

British Catalogue of Films for Universities

To assist the university teacher, who may have difficulty in locating films available for teaching purposes, the British Universities Film Council has issued a catalogue of some 1,400 titles classified according to the Universal Decimal Classification, together with an alphabetical index of titles (Pp. 166. Glasgow: British Universities Film Council, Royal College of Science and Technology, 1960). Besides a list of distributors there is appended a list of subject catalogues. The Catalogue has hitherto been issued as a card index, usually placed in the university library, but it is intended to reprint it in four or five years time. It is hoped that issue in book form, by widening its distribution, for example, so as to permit copies to be available in departments, will both stimulate the use of films in universities and comparable institutions and engender interest in expanding the catalogue. Suggestions as to films which might be included in future supplements are invited. Issue of the Catalogue in separate parts is contemplated and this should be an advantage to many users, particularly with further expansion of the Catalogue.

New British Oceanographic Research Ship

PLANS for a new research ship to replace R.R.S. *Discovery II*—now 31 years old—are almost complete, and it is hoped shortly to invite tenders for her construction by the early summer of 1962. The great advances in oceanography in the past decade have shown that there is a real need for a comprehensive research ship capable of working in any ocean, and in almost any weather. The demands for laboratory and ancillary spaces, together with present-day requirements for crew accommodation, alone necessitate a ship larger than *Discovery II*, and, as the life of a research ship may well be 25–30 years, allowance must also be made for future needs. The suggested design envisages a ship with an overall length of 260 ft., a beam of 44 ft. and a displacement of about 2,800 tons. Laboratory and other scientific spaces will occupy some 3,000 sq. ft. and will provide facilities for marine physics, biology, chemistry, geology, geophysics and meteorology. Diesel-electric drive, with a single screw, will allow a service speed of 10 knots and a range of about 15,000 miles. Special attention is being paid to the provision of adequate supplies of electric power, not only now but also in the future. Deck machinery will include separate winches for coring, trawling and all normal oceanographical work. Other special features are the bow propulsion unit, for giving sideways thrust, and an open trunk through the centre of the ship through which experimental apparatus can be lowered.

Postgraduate Education for General Practitioners

A RIDDLE which puzzles all those concerned with the maintenance of standards of general practice is why more than 70 per cent of the general practitioners in England and Wales undertake no form of postgraduate education. Some general practitioners feel that they can keep up to date by their contacts with fellow-practitioners or consultants or by reading. Others think that the postgraduate courses offered to them are not satisfactory. There are many, however, whose reasons for avoiding postgraduate courses are far from clear, and, to help them, a conference was arranged by the British Postgraduate Medical Federation and the Metropolitan and Home Counties Faculties of the College of General Practitioners. The eight opening papers of the conference have now been published in a recent issue of *The Practitioner* (185, No. 1109 ; November 1960). With the exception of Dr. H. G. McGregor, who is adviser on postgraduate education to the South-eastern Metropolitan Hospital Region, the contributors are all general practitioners. The papers represent an authoritative review of what general practitioners feel they require if postgraduate courses are to be of real value. A general conclusion is that the formal lecture should now have little place in the education of the general practitioner, who should play an active part in all postgraduate courses.

History of Science in Poland

IN December 1958, a plenary meeting of the History of Science Committee of the Polish Academy of Sciences decided that a concise outline of the history of science in Poland should be prepared. In character and level, it is to be a university text-book, emphasizing general aspects of science, conditions of development, organization of scientific work, and the trends in scientific thought which are common to various disciplines. Specific sections will be devoted to the development of various branches of science and the more eminent personalities and their work in Poland. The sections devoted to specialized subjects in particular periods are to be as succinct as practicable, and to embody the maximum possible volume of information. A tentative plan is discussed in the January–March issue of *The Review of the Polish Academy of Sciences* (5, No. 1 (17) ; 1960).

Salvage : Waste or Wealth

ALTHOUGH it is well known that one industry's waste may be the raw material for another, it is equally well recognized that many firms have found themselves in difficulties because their waste costs have been too high and because they have been unable to make a saleable commodity from their wastes. In the first of a new series designed to be of practical use to industrial management, the Department of Scientific and Industrial Research has produced a booklet which, properly used, could mean the difference between success and failure for many struggling organizations. The booklet, "Waste or Wealth," by Clement Brown, is a collection of stories which show how, by research or technical aid, many industrial wastes are being turned to profit (Pp. 27. London : H.M. Stationery Office, 1960). There are still serious cases of unclaimed wastes—several million gallons of paint are lost each year during spraying ; little attempt is being made to make use of scrap leather and a fresh application is needed ; a new use for sugar, now produced in excess of demand, is being investigated and could form the basis of a detergent. The booklet's stories of success should spur the inventiveness of management. It may be obtained free of charge from the Department of Scientific and Industrial Research Lending Library Unit, 20 Chester Terrace, London, N.W.1. Readers will wish to commend the Department for the earthy and practical way in which the booklet has been presented and for choosing Peter Kneebone to add those humorous illustrations which tell vivid stories without words.

The British Society for International Understanding

THE annual report for 1960 of the British Society for International Understanding, presented to the twenty-first annual general meeting on November 16, 1960, notes that the Society has now published some 600 British Survey pamphlets (Pp. 10. London : The British Society for International Understanding, 1960). These pamphlets have been written by experts and each is devoted to the distinct characteristics of a single Commonwealth or foreign country or to some well-defined international topic. Much of the energies of the staff is now directed to building up and maintaining the Atlantic Treaty Organization and its United Kingdom branch, the British Atlantic Committee. Lord Bessborough, the chairman of the governing body, has suggested that the time has come to give the same special attention to the cultivation of good relations and mutual understanding between Britain and the emergent nations of Africa. The report includes details of publications during the year and of the activities of both the Atlantic Treaty Association and the British Atlantic Committee.

Aeronautical Research in Australia

THE annual report for 1958–59 of the Aeronautical Research Laboratories of the Australian Defence Scientific Service has recently become available (Pp. 39. Melbourne : Department of Supply, Aeronautical Research Laboratories, 1960). The report is a well-illustrated booklet of thirty-nine quarto pages which record recent progress in research and development, and is refreshingly devoid of 'propaganda'. The research undertaken is relevant either to Australian and Commonwealth defence, or to Australian industry and national development. It is distributed between the various divisions, aerodynamics, structures, materials and mechanical engineering, and covers a very wide range of problems. Since guided weapons are finding wider and wider application and since Woomera, the Commonwealth test-range, is at hand, there is great emphasis on missiles and missile propulsion. A co-ordinated team specializing in this field is suggested. Nevertheless, there is much that concerns current military and civil aircraft. Some interesting work on the fatigue life of aircraft structures is reported—including an investigation aimed at providing a method for the experimental verification of the 'fail-safe structure' so often claimed by designers. There is also the work of the human engineering group on the piloting problems during landing. In an appendix are listed 44 reports and notes dealing with major research projects and the 36 technical memoranda on minor and *ad hoc* investigations. Altogether an interesting year's work.

Rock Carvings in Central Australia

THE October issue of *Man* contains an interesting article recording some simple rock carvings at

Ewaninga in Central Australia by C. P. Mountford, who has done so much work on this subject. The carvings are made by a pocking technique and consist of rough drawings of stylized figures and signs, consisting of spirals, circles, arrows, etc. The local native inhabitants apparently know nothing about them, and indeed frequently had not realized that they were the works of man. Rock carvings do not occur everywhere in Australia. They have not been found along the southern and south-eastern coasts, but have been found in Tasmania. In Central Australia they have been known for a long time; Mountford records that Miss Helen Teague gave him some photographs of carvings near Glen Helen as long ago as 1937. The signs are very varied in type, but are, of course, nowadays inexplicable.

Fertility of Ewes

Margaret Ryle, of the Department of Physiology, University of Queensland, has investigated the relationship between high temperature and vitamin A metabolism and the fertility of ewes (*Austral. J. Science*, 23, No. 2; August 21, 1960). The gross effects of vitamin A deficiency have much in common with those of high environmental temperatures. Partial inhibition of follicle development and of œstrus have been reported as a result of vitamin A deficiency in rats and rabbits and of high temperature in rats. Some of the available data on sheep suggest a similar vitamin A effect; the adverse consequences of high temperatures on conception-rate in sheep are reported by several authors. Increased embryonic mortality is described in rats, rabbits and sheep as a result both of vitamin A deficiency and high environmental temperatures. Vitamin supplements have been shown to reduce fœtal resorptions in heat-treated rats. Failure of normal development of the maternal uterine epithelium as the immediate cause of fœtal mortality is indicated both in vitamin A deficient rats and in heat-exposed rabbits. Changes have been described in the pituitary and thyroid glands both of vitamin A deficient animals and of animals exposed to high temperatures, but strictly comparable studies are required to determine whether or not they are of the same nature. In general, only further experimental work can decide whether the similar consequences of the two types of stress are merely coincidental, or whether they have a casual connexion such as the one suggested.

Forthcoming International Scientific and Technical Conferences

A list, No. 28, has been issued by the Department of Scientific and Industrial Research entitled *Forthcoming International Scientific and Technical Conferences*. The list, covering the period January 1961–December 1962, amends and amplifies the information contained in the previous lists on all scientific and technical conferences of an international character, held in the United Kingdom or elsewhere. The information is presented under four headings giving the subject of conferences, their dates, the convening bodies and/or organizers, and also the places where they are to be held. The subject index at the end does much to enhance the value of this publication. Copies can be obtained from the Department of Scientific and Industrial Research, Charles House, 5–11 Regent Street, London, S.W.1.

Announcements

Sir Brian Windeyer, of the Meyerstein Institute of Radiotherapy, Middlesex Hospital, London, has been appointed chairman of the Radioactive Substances Advisory Committee in succession to Sir Charles Darwin, who has resigned.

The Ministry of Agriculture, Fisheries and Food and the War Office announce that Brigadier A. H. Dowson, director of military survey, War Office, has been appointed director general of the Ordnance Survey, with the rank of major general, in succession to Major General L. F. de Vic. Carey, C.B., C.B.E., who is retiring on June 30.

The Jones–Bateman Cup, which is offered triennially by the Royal Horticultural Society for original research in fruit growing, has been awarded to Mr. A. Gavin Brown of the John Innes Institute, Bayfordbury, Herts, for his research work on the inheritance of susceptibility to diseases in apples and pears, the inheritance of shape, size and season of ripening in apples, and other matters relating to the breeding of fruits.

Mr. P. Lloyd, deputy director of research and development at the National Gas Turbine Establishment, has been appointed director general of engine research and development in the Ministry of Aviation

The International Measurement Conference, IMEKO 1961, is to be held in Budapest during June 25–July 1, 1961, and will be accompanied by an exhibition. Further information can be obtained from the IMEKO Secretariat, Budapest, 5 (P.O. 3).

Under the sponsorship of Nato, a summer school will be organized at the University of Ghent during July 24–August 5. The subjects to be considered are the electrical, optical and mechanical properties of lattice defects. Information can be obtained from Prof. W. Dekeyser, Laboratorium voor Kristallografie en Studie van Vaste Stoffen, Rozier, 6 Ghent, Belgium.

The first international congress on "Ergonomics" is to be held in Stockholm during August 20–23, 1961; the congress is being organized by the International Ergonomics Association. The programme will include the following subjects: speed of work and ergonomics; adjustment of work and working environment to the ageing population; ergonomical job analysis. Further information can be obtained from Prof. Sven Forssman, Svenska Arbetsgivareforeningen, P.O. 16120, Stockholm 16, Sweden, or the British representative on the provisional committee, R. G. Stansfield, D.S.I.R. Warren Spring Laboratory, Gunnels Wood Road, Stevenage, Herts.

Hansom Books, Ltd., under the title "Technical Books-in-Print", list and detail all technical and scientific books available in Britain, including books due for publication. In *Physics and Mathematics* of September 1960 the titles are arranged alphabetically under: physics—general; light and sound; electricity and magnetism; heat, radiation, thermodynamics; mechanics, dynamics, quantum theory; nuclear and atom physics; physics—miscellaneous; mathematics—general; applied mathematics; algebra; geometry, trigonometry; calculus, differential equations; statistics (Vol. 1, No. 8. Edited by W. G. Smith Pp. 60. (London: Hansom Books, Ltd., 1960.) 6*d*.). From the October issues the price of each part is increased to 9*d*. (11*s*. per annum by post). *Books in Britain* will be 3*s*. per copy, 42*s*. per annum.

NEW BUILDINGS AT THE FIRE RESEARCH STATION

By D. I. LAWSON

Joint Fire Research Organization of the Department of Scientific and Industrial Research
and Fire Offices' Committee

THE new buildings of the Fire Research Station of the Joint Fire Research Organization of the Department of Scientific and Industrial Research and the Fire Offices' Committee, which have a floor area of 30,000 sq. ft., were opened on November 4 by the Right Hon. Viscount Hailsham, Lord President of the Council and Minister for Science. This new accommodation, built at a cost of about £300,000, doubles the previous floor area and makes the Fire Research Station the largest and best-equipped organization of its kind in the world.

The scheme comprises a three-storey block (Fig. 1), 200 ft. long, 36 ft. wide and 33 ft. high, of laboratories and offices and two separate blocks of three 30-ft. high laboratories, each having a floor area of about 2,500 sq. ft., for hydraulics, thermal radiation and chemical engineering research.

A 7,500-gallon concrete storage tank is built in the floor of the hydraulics laboratory to conserve water returned from experiments. Two pumps are provided in the laboratory, one having a capacity of 1,000 gal./min. at a pressure of 121 lb./in.^{2} and the other a capacity of 250 gal./min. at a pressure of 156 lb./in.^{2}. These are used for testing equipment submitted by manufacturers, including alarm valves and sprinkler systems.

The radiation laboratory will be used for problems relating to ignition and spread of fire, and for this purpose a 4-ft. square gas-fired radiant panel has been installed at one end of the laboratory.

Explosion hazards of combustible dusts and the self-heating of unstable chemicals will be studied in the new chemical engineering laboratory.

The opening of the new buildings came nearly fourteen years after the formation of the Joint Fire Research Organization by the Department of Scientific and Industrial Research and the Fire Offices' Committee. One of the principal older buildings houses furnaces for examining the performance of structural elements under fire conditions, the largest furnace having a maximum gas consumption of 25,000 cu. ft. per hour; indeed, the maximum total gas consumption of the Fire Research Station is sufficient for a small town.

During 1958, a large laboratory, the models laboratory, 150 ft. long, 50 ft. wide and 40 ft. high, was added to the existing buildings for the purpose of studying the modelling of fires. This has a roof which will open over 24 ft. to release smoke and hot gases. A 12-ft. diameter fan, driven by a 200-h.p. motor, can be used to draw air transversely across the laboratory at speeds up to 30 m.p.h. over a working section 15 ft. long, 15 ft. wide and 10 ft. high.

The present staff totals 136, and the scientific work of the Station is organized into five sections.

Operational Research and Intelligence

Each year this Section receives from the Fire Service Department of the Home Office details of about 120,000 fires which have been attended by local authority fire brigades. After coding and analysing them, the Section produces annual statistical tables and notes trends in the incidence of fire, casualties and damage. Detailed studies are made of specific hazards—for example, fires caused by oil-burning appliances or by electricity. Investigations are also made into the effectiveness of equipment, such as sprinklers and fire-detectors. The work of this Section provides an essential background for planning the research programme as a whole and for the administration of fire protection.

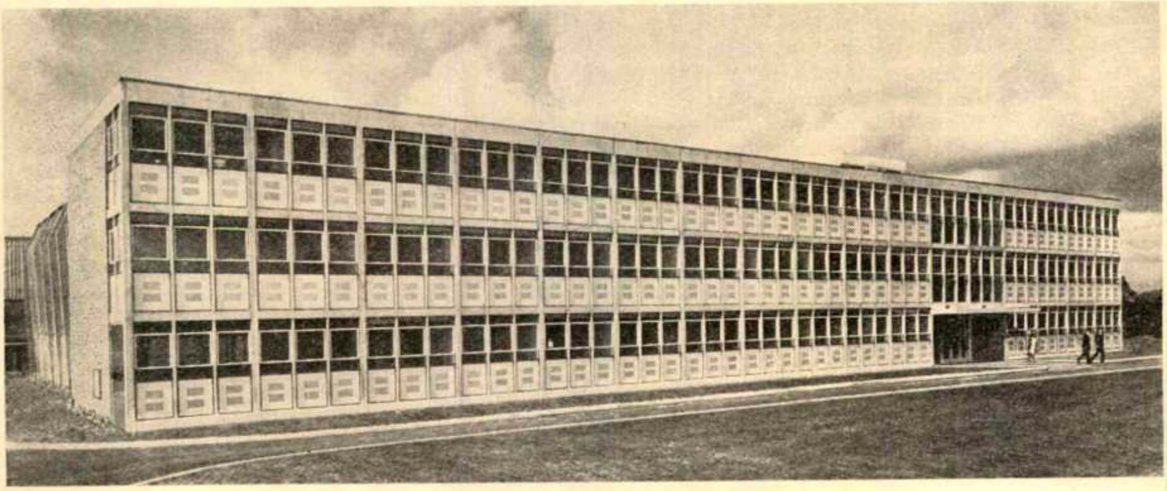

Fig. 1. The Main Building of the Fire Research Station, Boreham Wood, Herts

Ignition and Growth of Fire

In this Section physical aspects of combustion and heat transfer are studied. Techniques involving the use of models are being developed to determine the factors—particularly wind, ventilation, fuel, and shape and size of compartment—which control the growth of fire and its spread within and between buildings. The aim of this work is to provide a scientific basis for the fire grading and adequate separation of buildings. Ten laboratories in other countries are also co-operating on an agreed programme of experiments with models. The results of this international programme should lead to theoretical understanding of the progress of fires in buildings.

Further work is being carried out on the development of fires in the open, as this will have application both to forest fires and fires in built-up areas.

Building Materials and Structures

Since much of the total damage by fire occurs in buildings, this Section studies building materials under fire conditions to obtain basic data for the design and protection of structures which will help to control the development and spread of fire. It also performs, at the request of manufacturers, many tests on structural elements, and spread-of-flame tests on building materials. Its work is being used in the revision of building by-laws, and in the advice given to architects, builders, structural engineers and local authorities on appropriate types of structure and materials.

Chemistry and Chemical Engineering

This Section is concerned with industrial fires and explosions involving gases, vapours, dusts and unstable materials not classified as explosives. Working in close co-operation with the Factory Inspectorate of the Ministry of Labour, its aim is to assess hazards and devise precautionary measures. In collaboration with the Extinguishing Materials and Equipment Section, studies are made of the means of extinguishing fires in flammable liquids, the use of vapour phase inhibiting agents and the possibility of extinguishing fires in buildings by filling the interior with inert gas. Experiments with inert atmospheres are now being carried out with a specially adapted jet engine.

Extinguishing Materials and Equipment

The main purpose of this Section is to study the properties of, and methods of applying, extinguishing agents, for example, water, foam and dry powder. Models of rooms and buildings are used to learn more about the various mechanisms by which water puts out fires. This Section studies the automatic detection of fires and the performance of detection systems. It carries out tests—many of them for the Fire Offices' Committee—on equipment, including automatic sprinkler systems, portable fire-extinguishing appliances, and automatic fire alarms. In co-operation with official bodies and manufacturers, the Section assists in drawing up standards and specifications for such equipment.

In addition, the Fire Research Station has a library which provides an extensive coverage of fire literature as well as information and intelligence services.

FUTURE OF THE GENERAL DEGREE IN BRITISH UNIVERSITIES

DISCUSSIONS at the Home Universities Conference are not required to reach agreed conclusions. Had this been the case, the discussion on the "Future of the General Degree" during the recent Conference of the Universities of the United Kingdom, held in London during December 9–10, would probably have reached the conclusion that it is a good degree, but that it may have no future.

There were two opening speeches. The first was given by Mr. D. W. Reece, lecturer in humanity at the University of Aberdeen, who argued that the planned increase of student numbers to 170,000, which will bring the proportion of students to total population in England to the same figure as it now is in Scotland, must (since there are no grounds to suppose that the English are more intelligent than the Scots) lead to the entry of a large number of students of lower calibre than hitherto, who will need easier courses of instruction. He assumed that the general degree in England can be equated to the ordinary degree in Scotland, and that there will be an increasing need for it, as providing a suitable education for the less-able student.

The second opening speech, given by Prof. F. C. Frank (professor of physics, University of Bristol), dealt with the general degree with honours provided by most of the English universities, and in particular with the general degree of B.Sc. He took the opportunity to direct attention to the appalling complexity and disparity of terminology employed by various universities in describing the courses they offer. But while the general honours degrees are of various structure from university to university, broadly speaking they differ from special honours degrees mainly in the third year of study, which in a special degree is devoted to a single subject, whereas it still covers two or more subjects in a general degree.

The distinction between the special and the general degree ought not, Prof. Frank said, to be regarded as one of merit. He gave it as his considered judgment that in practice it is harder to get a first class in the general than in the special degree, though easier to get a poor second or a third class. It is judged to be a suitable career education for school-teaching, a course of education suited to the type of mind which is less happy concentrating on a narrow front, and a more balanced course for those whose intended future is a non-academic one (whereas the more academic will yet have time to take up their various academic subjects in turn). In short, he considered it a suitable education for a Cabinet Minister.

Judging from newspaper reports of the discussion, it is probably the former conception of the general degree which appears more realistic outside university circles. This view was not without support at the Conference, but most speakers concerned themselves with the general honours degree: its merit was confirmed, but its name was brought in question. The outstanding argument which can be made against the type of education given in a general degree was not brought into the discussion. This is Dr. C. R. Burch's principle, the broadening effect of specialization—if one studies chemistry, for example, superficially, it is chemistry only; the deeper one studies it, the more one is forced to discover its connexions with other subjects.

In the view of the University of Southampton, expressed by Prof. H. C. Baldry (classics), the general honours degree, under that title, has no future at all. It carries from the past (meaning, for Southampton, the period when it was under the tutelage of London) what according to this view is an indelible association with mediocrity. Southampton, in its regulations governing admissions since 1959, has abolished the general degree, but at the same time has provided a flexibility in the make-up of the course for an honours B.Sc. so that it is possible for it to be essentially similar to one of the less-wide general B.Sc. degrees, such as that provided at Bristol. Prof. G. Gee (Sir Samuel Hall professor of chemistry, University of Manchester), supporting the view that the choice between specialization and a wider curriculum is quite distinct from the need to provide courses at different intellectual levels, advocated a single B.Sc. certificate, with the appropriate class of honours, and the subjects studied, and for what periods, separately recorded on the certificate.

Against this view we have the policy, implied by the latest University of London regulations, and made explicit by Prof. J. G. Semple (professor of mathematics, King's College), that the general degree serves a dual purpose, being in its honours classes fully equal, and perhaps in the first class a shade superior, to the special degree, but in its pass category offering an easier route to a degree for the less-able student.

This is indeed how it tends to work out in practice, but it is a matter for urgent decision whether this dual purpose use is compatible with a proper recognition of the status of a good general honours degree. Prof. Frank, in his reply, considered that this was only possible if one made a strictly parallel division into general honours, general ordinary, special honours and special ordinary, even though ordinary specialists are the type of graduate which universities are least desirous of producing.

A distinct but important subject was brought into the discussion by Prof. W. F. W. Wynne-Jones (professor of chemistry, King's College, Newcastle on Tyne), namely, the danger that a general honours student may be nobody's baby. He suggested that interdepartmental professors—professors without portfolio—could take responsibility for these students, and Prof. Frank in his reply remarked that such interdepartmental professorships are being increasingly established in the United States, mainly to provide liaison between departments at the research-level. It was interesting to see in Prof. Wynne-Jones's proposal the way in which such interdepartmental professors could be given the opportunity to involve themselves with undergraduates.

F. C. Frank

THE UNIVERSITY GRANTS COMMITTEE

ANNUAL REPORT

THE annual returns from universities and colleges in Great Britain in receipt of Treasury grants for the academic year 1958–59, now covering 21 universities and 3 colleges, issued by the University Grants Committee, is this year prefaced by a somewhat longer summary of the Committee's major activities since June 1959*. Much time was given to consideration of possible changes in the university population during the 'sixties and 'seventies; and, following the Chancellor of the Exchequer's authorization in January 1960 to discuss with the universities the practicability of providing 170,000–175,000 places by the early 'seventies, the Committee asked the universities to consider what further expansion in student numbers they were prepared to contemplate by that date, together with their requirements for new accommodation. On the basis of subsequent discussions with the universities, the Committee has prepared a report for the Chancellor of the Exchequer. These discussions indicated that the existing universities, including the newly founded University of Sussex, could not of themselves alone provide a sufficient number of places, and that some new institutions were needed. The Committee therefore recommended to the Chancellor in March that immediate steps should be taken to encourage the establishment of new universities at Norwich and York, and authority to initiate such foundations was given in April.

After consultation with the Committee of Vice-Chancellors and Principals, the Committee has advised that these new institutions, which are to be multi-faculty, should have powers from the start to grant both first and advanced degrees, to set their own entrance requirements and to devise their own curricula. It is proposed that the safeguards previously achieved by close association with an existing university institution should be secured for a period by means of an Academic Advisory Committee consisting of members from existing universities and, possibly, with lay representatives. Possible means of assisting St. David's College, Lampeter, were explored during the year; a scheme has since been approved.

An independent sub-committee, with Sir William Hayter as chairman, was appointed to review developments in the universities in the field of Oriental, Slavonic, East European and African studies, consequent on the recommendations of the Scarbrough Commission in 1947, and to consider and advise on proposals for future developments.

An inquiry concluded during the year into the success or failure of students entering universities in October 1955 to study arts, pure science, technology, agriculture or forestry, showed that the proportion who left without success on account of academic failure was only 9·9 per cent, compared with 11·3 per cent in an earlier survey in 1952.

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* University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1958–1959. Pp. 58. (Cmnd. 1166.) (London: H.M. Stationery Office, 1960.) 4s. 6d. net.

The Committee has continued to give thought to ways and means of ensuring the maximum economy in the expenditure of capital funds, and standards of accommodation of various types, based on analyses of comparable buildings, on which universities can base their applications to the Committee for grants for new accommodation, have been suggested. The Committee has also considered and adopted new methods, based on cost analyses, of assessing grants for halls of residence and for science buildings; the new system of assessing grants for science buildings distinguishes between the cost of the building with its basic requirements in the way of heating, lighting and ventilation and its special requirements in the way of services, which may vary greatly.

The number of full-time students in British universities increased further to 100,204, compared with 95,442 in 1958–59, and statistics collected in October 1959 showed a university population of about 102,000. There were 6,084 full-time and 2,270 part-time students from overseas within the British Commonwealth, and 4,116 full-time and 1,944 part-time students from foreign countries; for 1957–58 the figures were 6,180 and 2,208 for the Commonwealth, and 3,982 and 1,904 for foreign countries. Of full-time men students, 36·8 per cent were in arts, 24·2 per cent in pure science, 19·5 per cent in technology, and 12·5 per cent in medicine; these figures compare with 36·4, 23·7, 19·0 and 13·7 respectively, in 1957–58. For full-time women students, the corresponding figures for 1958–59 are 62·6, 20·7, 1·0 and 11·9, respectively, and for 1957–58, 63·5, 20·0, 0 8 and 12·1, respectively. Full-time advanced students of pure science numbered 4,071 (34·9 per cent); of technology, 2,125 (18·2 per cent); and of medicine, 900 (7·7 per cent); 3,185 students were taking post-graduate teacher-training courses.

Of the full-time students, 80,805 were reading for a first degree, 3,928 for a first diploma, and 14,839 were engaged in research or other advanced work; the corresponding figures for 1957–58 were 76,687, 3,937 and 14,069, respectively. Of the new full-time students, 24·1 per cent were in pure science, 16·2 per cent in technology and 8·9 per cent in medicine; in 1957–58 the corresponding figures were 24·0, 15·4 and 9·1 per cent, respectively.

The proportion of assisted students was 80·5 per cent compared with 79·2 per cent in 1957–58 and 71·9 per cent in 1953–54, ranging from 92·4 per cent in Wales, 87·5 per cent in English universities, excluding Oxford, Cambridge and London, to 70·6 per cent for Scotland. Full-time teaching and research staff increased to 10,823, compared with 10,542 in 1957–58. The proportion of full-time students residing in colleges or halls of residence was 26·6 per cent, compared with 26·4 per cent in 1957–58; 49·6 per cent were in lodgings and 23·8 per cent at home, but these proportions vary widely between men and women and between institutions.

Of the recurrent income of £52,273,306 (an increase of £2,855,004 in 1957–58), £36,448,118 was from Parliamentary grants (69·7 per cent), £5,739,316 (11·0 per cent) from fees, £1,795,344 from endowments, £1,500,918 from local authority grants, £479,093 from donations and subscriptions, and £3,657,530 (7·0 per cent) from payments for research. Non-recurrent grants in respect of capital expenditure amounted to £16,554,483, compared with £11,816,479 in 1957–58; and of the recurrent expenditure of £51,526,275—an increase of £3,191,222 on 1957–58—6·8 per cent was spent on administration, 44·5 per cent on salaries and superannuation of teaching staff, 11·0 per cent on departmental wages, 13·2 per cent on departmental and laboratory maintenance, 3·4 per cent on repairs and maintenance of buildings, and 9·7 per cent on rates, insurance, heating, lighting, etc. Expenditure on libraries, at £1,979,945, remained at 3·8 per cent of the total.

WIND TUNNELS AND FLOW VISUALIZATION

THE Fluid Dynamics Panel of AGARD held technical sessions at Istanbul, Turkey, during October 3–4. Fifteen papers were presented, the two main topics being the 'industrial' use of wind tunnels, and flow visualization. The term 'industrial' used in this context means 'non-aeronautical'; and these topics were chosen for this particular meeting because of the special requirements of the host nation, which has little aeronautical industry. The meeting also presented a rare opportunity for workers in a field of aerodynamics which, while it does not usually receive the consideration of bodies concerned with aeronautics, is not afforded much prominence in the proceedings of the various engineering institutions to which the work is relevant.

The titles of the ten papers presented on industrial aerodynamics showed a similarity which was reflected to a large extent in the contents of the papers. Nevertheless the papers revealed a very wide diversity of problems, ranging from work on insect flight (using live subjects) to that of the aerodynamic oscillations of long suspension bridges. The introductory paper by Mr. C. Scruton (United Kingdom) gave a brief review of the work carried out at the National Physical Laboratory. This was classified under three main categories: (1) the study of flow problems; (2) the assessment of the steady-wind forces and pressures on vehicles, ships, buildings, etc.; (3) the oscillatory effects of wind on structures. The techniques used and the conditions for similarity of behaviour between the model and the full scale were briefly indicated. In the first category most of the work was concerned with the flow over the decks of ships, and with pollution from the effluent of industrial chimneys, either with regard to the adjacent buildings or to the surrounding countryside. The oscillatory effects of wind were dealt with at somewhat greater length, with particular mention of the oscillations which can be induced by an air-stream on suspension bridges or tall stacks, and in tube arrays, such as are found in heat exchangers. It is now usual to carry out aerodynamic stability tests in connexion with all major suspension bridge projects, and, for this purpose, it has been shown that costly and time-consuming tests of aeroelastic models of the complete bridge are unnecessary and that the simple and cheaper 'sectional' model technique, in which a model of a representative length of the suspended structure is mounted on springs in the wind tunnel, is adequate. These tests have been usually carried out in a steady windstream, but on occasions it has been necessary to investigate the possibility of buffeting oscillations of the bridge arising from its immersion in the eddying wake of a nearby bridge. The wind-excited oscillations of tall circular stacks are usually in bending modes transverse to the wind direction, and are due to the alternating 'lift' forces produced by the shedding of vortices from alternate sides of the section. Whether or not a stack oscillates depends on the natural frequency (on which depends the critical speed for instability) and on the structural damping. Artificial structural damping has been used to suppress the oscillations, but it may be that a more satisfactory solution is to be found in modifications to the cross-sectional shape which destroy the aerodynamic excitation. One such device which has proved successful was developed at the National Physical Laboratory, and consists of a three-start strake wound helically round the surface of the stack. This has the effect of destroying the regular periodicity in the formation of vortices. The top of a stack may be forced also into oscillations as an elastic ring by the action of the vortices. A ciné-film was shown which records such an occurrence at a power station in England and provided a graphic illustration of this effect.

The paper by Mr. P. J. Pocock (Canada) provided a most complete review of the industrial uses to which wind tunnels have been put in various countries, together with a comprehensive bibliography of no less than 385 references. The range of subjects investigated is very wide: meteorology, water conservation, soil erosion by wind, the mechanics of wind-driven snow, dust transport, are included as well as those discussed by Scruton. The aerodynamics of sails is now receiving attention at the University of Southampton. Pocock also referred to the work on animal flight which has been carried out in wind tunnels. This ranges from the testing of dead or sculptured birds to the more intriguing measurements on live insects which were tethered on a delicate and intricate wind-tunnel balance and their performance in flight measured as they flew against the horizontal wind.

In his paper on the aerodynamic problems of motor-cars Prof. H. Schlichting (Germany) demonstrated the very considerable saving in fuel consumption which has been achieved on the Volkswagen lorry by a modification to the frontal shape suggested by wind-tunnel tests. Reference was also made to the use of such tests to obtain data required for assessing the directional stability at high speeds of the Volkswagen private car, and of the stream-lined *NSU* motor bicycle which attained the world's speed record.

Prof. G. H. Strom (United States) discussed several interesting aspects which had been studied at New York University. With the reduced linear scale necessary, the representation in a wind tunnel of portions of the atmosphere, to include motions of mechanical and thermal origin, was a complex problem. The determination of the appropriate scale criteria needed further development, particularly when convective air currents were significant. Prof. Strom mentioned experiments in which powdered borax had been used to simulate snow in tests of snow drifting, and tests to study the production of capillary waves on a water surface.

A brief paper by Mr. H. Petersen (Denmark) described a wind tunnel especially suited for work in industrial aerodynamics. The tunnel design was based on the model-law proposed by Dr. Martin Jensen to provide the correct simulation for phenomena in which the influence of the vertical gradient of wind speed near the ground is significant.

The range of the problems investigated in wind tunnels was extended still further by the subsequent speakers. At Lille, A. Martinot-Lagarde (France) has studied mine ventilation, the fluid-flow in heat exchanges, and, more remarkably perhaps, the drag of fishing nets. A surprising result of the last-mentioned tests was that the drag of a net of nylon material was only 0·7 that of a net of similar configuration in cotton.

An open-jet type wind tunnel at the National Luchtvaarlaboratorium has been used extensively by Mr. N. Feiss (Netherlands) for investigating a wide variety of industrial problems. Some interesting techniques have been developed for predicting the nuisance caused by the deposition of rain and snow on the windscreens of vehicles.

Profs. R. Clay Porter and F. K. Boutwell (United States) have used the low-speed wind tunnel at the University of Michigan for some detailed work on air pollution from industrial effluents. An investigation at present in progress concerns natural draught cooling towers. It is claimed that such work has resulted in considerable financial savings by the industry.

The subject-matter of the two remaining papers, by Mr. R. F. Creasey and Mr. K. Emslie (United Kingdom), "Industrial Uses of Wind-tunnels with Particular Reference to Wind Machines", and by Mr. L. E. Leavey (United Kingdom), "Wind-tunnel Tests to determine the Efficiency of a Proposed Extension to a Welding Shed on Suppressing Draughts", may be gathered from their titles. It may be noted that these were the only papers to describe work of a non-aeronautical character carried out in the wind tunnels of aircraft manufacturing firms. Such investigations have been mostly carried out either by the universities or by national scientific establishments. The subjects treated and the techniques described in the papers from the several countries were broadly similar. However, there were sufficient differences to avoid repetition, and to make all the papers very valuable and worth-while contributions to an art which is rapidly increasing in its importance and in its application to engineering design.

The first of three papers on flow-visualization was presented by H. Werle (France), and described a number of facilities, using water as the fluid medium, which allow qualitative work to be carried out on problems of air-flow. These facilities included a narrow tank for viscous-flow work, a water tunnel for incompressible flows, and a free-surface tank for the supersonic analogy. The paper was given with the aid of an excellent ciné film in colour, the use of dye streamers of different colours enabling three-dimensional flows to be followed very easily. Among the phenomena shown in this film were the flow of swept wings in which the leading-edge vortices 'burst' at a little distance downstream, the effect of 'blowing' over flaps and ablation effects on a bluff body. For the latter experiment the body was moulded in ice, but no similitude with the full-scale phenomenon was claimed.

Mr. R. L. Maltby (United Kingdom) based his presentation on one of five papers which have been prepared for an AGARDOgraph on flow-visualization, and which describes the methods used in British low-speed wind tunnels. In addition to the usual 'smokes', mention was made of the use of soap bubbles. Condensation trails can form a natural method of visualizing the leading-edge vortices from swept wings when the vorticity is of sufficient strength to produce the condensation.

Moving from methods suitable for use in low-speed tunnels to those for high-speed flow visualization, J. L. Solignac (France) described interferometric apparatus used at the Office National d'Etudes et de Recherches Aéronautiques. One of these employed split-beam interferometry, whereas the other, which was developed there, was a schlieren-interferometer system using polarized light. As compared with the ordinary schlieren system, the latter method was claimed to have the advantages of simplicity as well as of the ability to make quantitative measurements.

C. SCRUTON

PRIMARY AND INITIAL EFFECTS OF IONIZING RADIATIONS

PROF. G. M. FRANK organized in Moscow during October 18–22, on behalf of the Academy of Sciences of the U.S.S.R., an international symposium which was sponsored by Unesco and the International Atomic Energy Agency. Half the thirty participants came from countries other than the U.S.S.R. As the contributions ranged widely and encompassed several disciplines, the classification under the headings chosen for this report must be somewhat arbitrary.

Interference with Intracellular Structures

The concept that the initial radiochemical reaction leading to the death of a cell is brought about by interference with the intracellular organization of the cell found support from many of the participants. In the first of the scientific papers, P. Alexander (London) reviewed the chemical data concerning damage to proteins and nucleic acids by ionizing radiation, and concluded that reactions of this kind, although they occur in cells, as do many others, did not meet the requirements for the radiochemical events that initiate cell death. He suggested the phospholipids of the intracellular membranes as a more probable site. Since one of the most frequent and earliest biochemical lesions following irradiation is an increase in enzymatic activity, it had been proposed by Bacq and Alexander (enzyme-release hypothesis) that radiation interferes with the structures within the cell that are responsible for its essential compartmentalization. G. M. Frank (Moscow) provided a striking demonstration of an increase in metabolism within an hour of irradiation by showing that the time taken for oxygen-levels in the brain to return to normal after a short period of inhalation of pure oxygen was less in an irradiated animal. The same technique also revealed that radiation immediately interferes with the homeostatic maintenance of oxygen-levels in tissues.

L. A. Stocken (Oxford) and M. Errera (Brussels) described their experiments on the immediate effect of X-rays on the metabolism of the nuclei of rat thymocytes. Stocken concluded that low doses produce local disorganization of the nucleus, which alters binding sites, and he outlined his current research programme directed to discovering the factors that maintain the nucleus in an organized state. Changes in the structure and behaviour of cytoplasmic fine structures within minutes of irradiation were seen by M. N. Meissel (Moscow) using fluorescent vital staining and by A. Shabadash (Moscow) with histochemical techniques. O. Hug (Munich) reported that smooth muscle was unexpectedly radiosensitive, and contracted with a dose of a few hundred röntgens so long as this was given at a sufficiently high dose-rate. He interpreted this effect as a temporary change in permeability of the nerve (or muscle) membrane.

A. M. Kuzin (Moscow) observed mitotic inhibition in the tip of the bean root (*Vicia faba*) although only a leaf had been irradiated. An antimitotic substance could be extracted from irradiated leaves and on purification was shown to be a soluble oxidation product of a polyphenol (a quinonoid substance). Evidence was found in support of the view that the active substances were formed as the result of the release (activation ?) of enzyme peroxidases from cytoplasmic organelles.

A. J. Passynsky (Moscow) was led by general kinetic considerations to the view that death of a cell by radiation was due to interference with the orderly sequence of biochemical events due to damage to the interphases at which they occur. Experiments in a model system illustrated the breakdown of membranes by 20,000 r. of X-rays. Particles of an enzyme were coated with a layer, approximately 100 Å. thick, of ribonucleic acid and then suspended in a solution of substrate. Enzymatic activity was accelerated on irradiation because the permeability of the ribonucleic acid film was increased. N. N. Doemin (Moscow) carried out a very detailed study of the effect of radiation on the lipids and lipoproteins in different sub-cellular fractions (microsomes, mitochondria); surprising differences were observed in different organs of the rats, though in every case early lesions among lipids were observed.

B. N. Tarusoff (Moscow) has found that the concentration of lipid peroxides in tissues of mice increases with time after irradiation, which initiates the oxidation chain. A correlation was seen between the peroxide-level and the time of death of the animal, and Tarusoff supports the view advanced some years ago by J. St. L. Philpot that toxic peroxides contribute to radiation death in mammals.

Genetic techniques using 'sexual' transfer between biochemically deficient mutants were used by H. Marcovich (Paris) to show that the killing of *E. coli* (that is, the loss of ability of the cells to proliferate indefinitely and form colonies) could not be attributed to a dominant lethal mutation. K. Tobias (Berkeley), on the other hand, argued that killing of yeast and the very complex relationship between ploidy and radiosensitivity could be adequately explained as the combined action of dominant and recessive lethal mutations.

A comprehensive mathematical formulation of the processes leading to cell death was presented by A. V. Lebedinsky (Moscow), who considered the simultaneous occurrence of repairable interference with metabolism and irreversible blocking of mitosis.

Modification of Radiation Damage

Death of a cell, rate of mitosis, chromosome damage, mutation production can be altered by a variety of post-irradiation treatments in unicellular organisms and some plant materials. N. I. Shapiro (Moscow) has now found that chromosomal damage in Ehrlich mouse ascites cells irradiated *in vitro* and then injected back into the animal can be substantially reduced if the cells are suspended in a medium containing arginine for 1 hr. after irradiation. A. Hollaender (Oak Ridge) reported that genetic damage in *Paramecium* could only be repaired if the post-irradiation treatment was begun before chromosome duplication had occurred. Shapiro's data could bear a similar interpretation. Powerful synergism in the amount of chromosome damage was found at Oak Ridge if a dose of X-rays was followed by ultra-violet irradiation within a comparatively short time. F. Herčik (Brno) demonstrated that the treatments that result in the recovery of *Paramecium* also lead to recovery of the phage-synthesizing capacity of *E. coli* in spite of the fact that doses of the order of 5×10^5 r. are needed to inhibit this synthetic process. Another Czech scientist, J. Soška, finds that the regeneration of bone-marrow *in vivo* is significantly increased by the administration of cytidylic and thymidylic acid, but not by purine nucleotides. Interference by radiation with deoxyribonucleic acid synthesis of bone-marrow cells *in vitro* is reduced by the addition of triphosphates to the medium.

The reduction of the killing action of X-rays for mammalian cells in tissue culture by fractionation had been interpreted as evidence that post-irradiation restoration processes occur normally. On repeating these experiments with α-rays from polonium, J. W. Barendsen (Netherlands) could find no evidence for recovery, suggesting an important qualitative difference between densely and sparsely ionizing radiations.

Radiation response can also be modified by treatments that have to be given before and during irradiation. L. H. Gray (London) provided further evidence that the radiosensitizing action of oxygen cannot be attributed to early post-irradiation metabolic processes, but that it must be connected with the immediate radiochemical events. The phenomenon is, however, far from simple, and B. N. Tarusoff (Moscow) has shown that there is an optimum oxygen concentration for sensitization in the case of yeast cells. If the amount of oxygen in the atmosphere exceeds 80 per cent the cells become less radiosensitive. This observation is interpreted as support for the view that a branched, oxidative chain-reaction is involved in the initiation of radiation damage.

The mechanism of radiation protective agents received much discussion. Z. M. Bacq (Liège) stressed that the almost universal protective action of cysteamine arose from the fact that this substance could protect by at least three different physicochemical mechanisms: radical capture, energy transfer and 'repair' by transferring a hydrogen atom to an organic radical. There was no reason for believing that in biological systems the same chemical mechanism was involved in every case. In mammalian systems radical capture or 'repair' seemed the most probable and the suggestion that cysteamine protected mammals by producing anoxia had to be rejected. This view was strongly supported by the extremely detailed investigations of E. Y. Grayevsky (Moscow). His group showed that the protective action of hypothermia, neurotropic substances and substances combining with hæmoglobin, but not of cysteamine, was directly attributable to a fall in oxygen tension in the radiosensitive tissues to less than half the normal value. L. H. Gray (London) showed that the protection of cell suspensions *in vitro* by aliphatic alcohols was also not related to the oxygen effect. Evidence that protection against the mutagenic action of X-rays is independent of protection against death of the cells was presented by A. Hollaender (Oak Ridge). 10^4 r. kills no cells of *Aspergillus terreus*, but the number of mutations produced by doses of this order is greatly reduced by cysteamine.

P. Alexander (London) interpreted his results on radiosensitization as showing that substances which are normally present in the cell can act as intracellular protective agents. Blocking of sulphydryl groups of lymphoma cells by iodoacetate was responsible for the radiosensitization of these cells by this substance. The great variation in the radiosensitivity of micro-organisms may in part at least be due to the presence of particularly effective intracellular protective substances; for example, carotenoid pigments.

Radiosensitivity of Biological Macromolecules

E. C. Pollard (Yale) described the use of radiation as an analytical method for determining the size and shape of functional units within cells. He deduced that the organelle responsible for protein synthesis was a unit having the weight of a microsomal particle, but with highly asymmetric shape. This quite unexpected finding receives striking support in recent electron microscopic studies on sections of frozen dried cells which have suggested to some workers that the microsomal particles can assume a sheet-like character. L. H. Gray (London) interpreted death of a bacterial cell as a single-hit target event, since he finds the dose-response curve to be accurately exponential over a vast range. On the other hand, J. W. Barendsen (Netherlands) finds that a target type interpretation cannot be applied to mammalian cells since the log (survivor) v. X-ray dose-curve never becomes truly linear and increases continually in steepness. K. Tobias (Berkeley) presented data showing that the determination of 'target sizes' was impossible even for isolated pure proteins, as there was interaction between particles.

L. A. Blumenfeld (Moscow) gave a detailed review of what can be learnt from the electron spin resonance patterns of irradiated proteins and nucleic acids and provided further evidence for his own well-known theories on the presence of conduction bands in native proteins. The use of electron spin resonance for discovering the nature of the initial lesion is very limited, as almost all cell constituents give rise on irradiation to radicals. All these radicals are seen by electron spin resonance, although many of them have no part in the biological effects. E. L. Powers (Argonne, U.S.A.) used the electron spin resonance method to good effect to confirm the presence of a number of different types of trapped free radicals which remain in the dry spore after irradiation. He observed that post-irradiation treatments with hydrogen sulphide and nitric oxide reduced the number of spores rendered non-viable, while exposure to oxygen enhanced damage. Electron spin resonance measurements indicated that these effects were due to reaction with trapped radicals. In swollen systems, such as vegetative cells, radicals have only a very short lifetime, and to be detected a 'flash radiolysis' technique has to be used. L. H. Gray described some results obtained in this way. Mrs. B. Hölmes (Cambridge) described experiments of J. S. Mitchell which revealed the existence in irradiated mammalian tissues of relatively long-lived excited molecules that emit ultra-violet light after irradiation.

PETER ALEXANDER

COKE RESEARCH

NOT all annual reports of technical, or even non-technical, activities provide an absorbing story of events of interest to others than the specialist. The sixteenth annual report of the British Coke Research Association, however, is noteworthy for a new feature in the form of a very clearly expressed appendix, in which an attempt is made to clarify the objectives of the programme of research*. Any likely developments that may point to the future importance of coal should be of interest to many engineers and technologists, and indeed to quite a number of laymen engaged in pursuits perhaps of greater philosophical calm. Particularly so must be the situation at the present time, when one hears so much of the potentialities of oil and nuclear fuels, and when hoping that the price of coal was tending to find a stable keel, one is startled by new economic storms. In a refreshing and lucid manner the research objectives of an important industry on which the future of steel and coal must depend to a substantial measure are set out in a manner that brings conviction of the care and thought given to their preparation.

The interested reader will learn that 40 per cent of the research effort of the British Coke Research Association is devoted to the improvement in quality and yield of metallurgical coke, and that a further 28 per cent is devoted to work designed to throw light on phenomena and factors that govern the efficiency of use of all types of coke.

The modern blast furnace requires a coke of consistent quality in respect of its physical and chemical properties. The Coke Research Centre at Wingerworth has its small experimental ovens and the industrial scale 10-ton oven in which the various factors may be worked out to enable the optimum conditions to be arrived at for the carbonization of a 'standard' coal. At the same time, attention is devoted to the yields and nature of the by-products from the point of view of the efficiency and economy of the coke-oven process in general.

In the scientific work on coke the more advanced techniques of physical science such as X-ray diffraction have long been used. The latest techniques, that are now finding a place in the elucidation of a workable understanding of the structure of carbon and the texture of coke, are concerned with magnetic susceptibility and microwave absorption. Perhaps the lessons of the value of the more abstruse techniques necessary in fundamental investigations are not lost in these days on the comprehension of the layman, even if the mysteries of the techniques themselves seem to provide rather heavy going. In the present instance the ultimate answer may well be the means of production of cokes, capable of superior performance in use, from supplies of coal of unexpected suitability.

In the programme of research two other broad fields of interest are considered: first, the magnitude and complexity of the coke oven effluent and atmospheric pollution problems; and secondly, new methods of making blast furnace coke. In the former, the problem becomes an issue common with many other industries, and it is thus only a part of a larger national issue being considered by the Department of Scientific and Industrial Research. In the latter the matter is at the design-study stage where the fundamental requirements of the problem have to be analysed, the estimates of the chances and conditions of success determined, and some practical course of research devised.

The report of the achievements of the year covers not only the work on the new full-scale test plant but also that of the fundamental and applied studies laboratories at Wingerworth, and the progress achieved by work in the universities, notably at King's College, Newcastle upon Tyne. In this last case the technique of electron resonance absorption has been used to follow in the course of coke formation the development of reactive centres, described as 'free radicals'. They are formed in coke at temperatures up to 800° C., and play a part in the fixation of sulphur as organic sulphur in coke. At the University of Leeds work on the flow of heat in the coke oven during carbonization has been prosecuted with the application of a digital computer. This work constitutes a further proof of the potentialities of the use in this manner of the unsteady heat conduction equation in the solution of practical problems.

Also of specific interest to scientist and technologist alike is the correlation found, almost to a surprising degree, between the results of the performance in carbonization from a 5-cwt., a 10-ton oven, and even a commercial battery. Practical trials on cupolas have settled the question of the effect of coke size in iron melting, and have shown that a 3–1 in. coke size may be used successfully in hot-blast cupolas.

A very wise decision has been not to allocate 15 per cent of the effort of the Association to any strictly defined objective. For is there not force in the argument that on the border of the unknown there is needed the keener search? Alike, as in diagnosis, the less definite the symptoms the greater the need for the sensitive touch!

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* British Coke Research Association. Sixteenth Annual Report for the year 1959. Pp. 40. (Chesterfield: British Coke Research Association, 1960.)

SOLID-STATE PHYSICS AT MELBOURNE

A SUMMARY of the proceedings and notes on the papers presented to the conference on solid-state physics held in Melbourne during August 17–21, 1959, under the auspices of the Australian branch of the Institute of Physics, has been published in the *British Journal of Applied Physics* (11, 137 ; 1960). The conference was the first of its kind to be held in Australia, and there was an attendance of about 130. Financial assistance in the form of grants was received from the Australian Atomic Energy Commission, the Commonwealth Scientific and Industrial Research Organization, the Australian Academy of Science, the Universities of Melbourne and New South Wales, and several industrial concerns. Forty-six papers were read and discussed at the conference, and the texts of a number of the original contributions have now been published in a special issue of the *Australian Journal of Physics* (13, No. 2*A* ; July 1960).

Prof. C. S. Barrett, of the University of Chicago, discusses the structure of the semi-metal bismuth at low temperatures, and describes a precision determination as a function of temperature of the displacement parameter of zone-refined bismuth containing less than 0·3 part per million of spectroscopically detectable impurity. The small but significant dependence on temperature at low temperatures disagrees with predictions from Blount and Cohen's theory and may signify a decrease in resistance to atom displacement from the simple cubic structure because of the thermal expansion of the structure.

Dr. J. J. Gilman, of the General Electric Research Laboratory, contributes an interesting paper in which he presents evidence to show that crystals exhibit a plastic resistance to dislocation motion. Direct observation of such resistance has been made with lithium fluoride and ferrosilicate crystals. The yield stresses and indentation hardnesses of many crystals can be correlated with their elastic moduli, and therefore the elastic modulus is one important factor which determines the plastic resistance. Another is the crystal structure type. Several papers deal with thermal transport in alloys, and individual papers with the nucleation and growth of ice crystals on crystalline substrates, X-ray measurements in metals, and the photographic process as a diatomic reaction.

SCIENTIFIC RESEARCH IN BELGIUM

THE annual report for 1959 of the Institute for the Encouragement of Scientific Research in Industry and Agriculture, Brussels, records research grants totalling 192,507,902 francs and grants amounting to a further 7,128,750 francs for special courses and travel ; 65 of the 79 grants for special courses were in chemical sciences and 7 in agricultural science*.

Of the research grants, one of 19,945,000 francs was for a systematic investigation of the pedological character of Belgian soils, and the completion of the pedological map of the country ; 13,220,000 francs were for the continuation of researches on the physics of the solid state in the laboratories of Prof. Dekeyser at the University of Ghent, of the Electrical Construction Factory at Charleroi, of Prof. Brasseur at the University of Liège and of the Gevaert Factory ; 11,200,000 francs to the Scientific and Technical Research Centre of the Metal Industry for research on the metallurgy of steel and special ferrous alloys, and a further 1,825,000 francs for soldering research ; 3,900,000 francs to the Carbochemical Society of Tertre for research on textile dyeing ; and 6,750,000 francs to the Gevaert Photoproducts Society for work on photographic dyes and processes of colour photography.

The Committee for the Application of Isotopical Methods in Agronomic Research received 9,485,000 francs for the continuation of researches on the utilization of radioisotopes, the Hainault Section of the National Centre for Metallurgical Research 7,770,000 francs for investigations on furnaces, 4,865,000 francs for its work on the continuous fusion of steel, the resistance of steel to brittle fracture, the improvement of converters, etc. ; and the General Metallurgical Society of Hoboken 7,560,000 francs for work on tantalum, niobium and their conversion to massive metal. Further subsidies of 2,905,000 francs and 4,455,000 francs went to the National Centre of Metallurgical Research and the Scientific and Technical Research Centre of the Metal Industry, respectively, for continuation of research on the mechanical properties of steel and improved methods of utilizing steel. The Liège Section of the former received a further 6,891,500 francs covering Belgian participation in the work of the International Committee for Research on Low Temperature Furnaces and 6,200,000 francs for continuation of research on cobalt. The Committee for the Study of the Nitrogen Problem received 6,520,000 francs for its studies on nitrogen in Belgian soils and on the fixation of nitrogen by legumes ; the Centre for the Biological and Colloidal Chemistry of the Soil, 6,395,000 francs for work on the formation of the organic matter of the soil and on the soil colloids ; the Research Centre of Gorsem, 5,500,000 francs for its researches on horticultural and agricultural diseases and pests ; the Scientific and Technical Centre of Belgian Textile Industry, 5,109,500 francs for new research on the manufacture of fibroplasts, on the carding of wool, on the utilization of isotopes for investigating the spinning of cotton and on the inter-relation between the properties of the threads and fibres of cotton ; the Biological Forestry Centre of Campine limbourgeoise, 4,800,000 francs for pedological, entomological, genetic and sylvicultural research bearing on the improvement of exploitation, conservation and natural regeneration of forests; and the Agricultural Physicochemical Centre, 3,960,000 francs for work on an improved method for the chemical determination of the nutritive elements in soil and the interpretation of the analytical results.

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* Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel. Exercise 1959. Pp. 237. (Bruxelles : Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1960.)

Grants of 3,155,000 francs and 2,670,000 francs went to the Scientific and Technical Research Centre of the Explosives Industry and the Centre for Research on Plant Hormones, respectively; 2,630,000 francs to the Centre for Research on Parasite Diseases of Domestic Animals; 2,400,000 francs to the National Committee for the Study of Fruit Cultivation for the equipment of two large conditioning chambers; 2,365,000 francs to the Centre for the Study of the Forest Soils of Ardenne and of Gaume for research on types of forest humus, and 2,240,000 francs to the Centre for Research on Diseases of Ornamental Plants. The Committee for the Study of Agriculture received a grant of 1,975,000 francs; the Plant Physiology Laboratory of the Gorsem Research Centre, 1,890,000 francs to extend its researches on the maturation of fruits and their conservation and studies on photosynthesis and on the influence of insecticides and fungicides on the physiology of the vegetables on which they are used; the Centre for Phytopharmaceutical Research, 1,836,000 francs for research on the utilization of phytopharmaceutical products; and the Committee for the Study of the Solid State, 1,650,000 francs for research on the production of durable magnetic materials.

Among other grants are 1,610,000 francs to the Pedological Service of Belgium, 1,412,000 francs to the Technical and Scientific Centre of the Wood Utilization Industry, 1,410,000 francs to the Committee for Strawberry Research, 1,350,000 francs to the Committee for the Study of Creep of Metals, 1,230,000 francs to the Belgian Centre for the Study of Corrosion "Cebelcor" and 1,080,000 francs to the Committee for the Scientific Study of Lactation.

MYOCARDIAL TISSUE CHANGES ASSOCIATED WITH RIGOR MORTIS

INITIAL tissue changes associated with rigor mortis do not appear simultaneously throughout the myocardium, their onset being earlier in some areas than in others; such changes result from biochemical modifications occurring after death in myocardial fibres, that is, post-mortem dephosphorylation of adenosine triphosphate and disappearance of creatine phosphate. The evidence from an investigation by Piero Gallo, of the Tumour Control Research Centre, Rome, indicates that the adenosine triphosphate and creatine phosphate content in the areas first involved by rigor mortis is significantly smaller than that found to be present in other tissue areas (*Cardiologia*, 37 Supp. 1; 1960). Since adenosine triphosphate and creatine phosphate represent the last link in the long chain of reactions involved in the production of chemical energy, the results enable the rate of this metabolic stage in different tissue areas to be evaluated quantitatively.

Far from being uniform throughout, the rate of energy production appears to be lower in areas where microscopic signs of rigor mortis begin, because these areas have the lowest content of adenosine triphosphate and creatine phosphate; the areas first involved by rigor mortis are segments averaging 20μ in length of cardiac muscle fibres, the whole width of which is affected.

The non-uniform distribution of adenosine triphosphate and creatine phosphate in the myocardium has been compared with the results of investigations concerning (*a*) the distribution of interfibrillar cytochondria, which is remarkably uniform; and (*b*) the diffusion speed of oxygen and energetic materials from the capillaries to the fibres, which varies in tissue areas.

These variations chiefly affect the aerobic phase of metabolism as well as the concentration of creatine phosphate and adenosine triphosphate, which is lower in more slowly oxygenated areas.

Analysis of these experimental findings enables the following conclusion to be drawn: the relatively small amount of adenosine triphosphate in some myocardial areas is not dependent on the distribution of cytochondria, but appears to be due to the fact that such areas are supplied with oxygen at a slower rate than the remaining tissue areas. The slow rate of gas exchanges in such areas seems to be responsible for a marked decrease in the yield of oxidative phosphorylation processes, so ensuring the transfer of chemical energy derived from breakdown and oxidation of energetic materials to creatine phosphate and adenosine triphosphate, which retain it and yield it to myocardial fibrils at the time of contraction.

In the rabbit, topical application of γ-hexane on the skin at a dose-level of 2 mgm./kgm. body-weight does not alter the course of myocardial tissue changes associated with rigor mortis, which exhibit a pattern almost identical to that observed in animals not treated with this insecticide.

BOWER-BIRDS

THE popular belief that the display of bower-birds is either indiscriminate or satisfies some conscious æstheticism has been questioned by Prof. A. J. Marshall, of Monash University (*Endeavour*, 19, No. 76; October 1960). He suggests that, remarkable as they are, the bowers and display paraphernalia of bower-birds are no more than an extension of the territorial and display impulses to be found in other birds. Avian display—visual and auditory—is usually associated with conflict and the establishment of territorial domination, pair formation, and seasonal gonad development; final synchronization of the sexual processes of the pair occurs when the environment changes seasonally to a state appropriate for successful reproduction. Only then will the female accept the male. All kinds of widely unrelated birds —for example, waders, grouse, manakins and lyre-birds—make simple display-grounds and posture on them during their sexual season. These arenas are evidence of convergent evolution.

In bower-birds a simple display came first. The ontogeny of the satin bower-bird's display can be seen in the increasing elaboration of its ritual as the individual gains in maturity and experience. By scattering a few fragments of blue glass under a wild fig tree on which the young were feeding, Marshall was able to stimulate them to fly down, to snatch up the glass, and to display even without a bower. Later the young green males would build a simple platform of a few twigs and display there with the accumulated coloured objects ; generally these would be stolen from neighbouring adult blue males and carried off to their bowers. As the young green birds grow older, they make low-walled avenue bowers, but before they themselves change colour at the age of five or six years to a mature blue-black they are capable of building a bower as elaborate as that of their elders.

Marshall sees the bower as a result of a displaced nesting-drive. The male, which takes no part in nest building, erects a structure on his display arena. The bower-birds have developed this display tendency—known in various other species—to an extraordinary degree ; some species select coloured objects in the image of male rivals and use them violently in a noisy display which attracts the female and deters interlopers. Strong intraspecific competition has been a powerful factor in the evolution of bower-bird display.

Much has been written about the supposed intelligence of bower-birds, but there is evidence that they are no more intelligent than other highly developed passerine species. Much, too, has been written of their alleged conscious artistry ; but their choice is essentially mechanical and unvarying. A species that uses greenish-yellow, for example, will never select green. None, so far as is known, ever varies its colour scheme.

RADAR ECHOES FROM THE AURORA AT 1,300 MC./S.

By DR. E. EASTWOOD, G. A. ISTED and J. D. BELL
Marconi's Wireless Telegraph Co., Ltd., Great Baddow, Essex

DURING the course of radar experiments directed primarily to the study of the movements of 'angels' and various types of cloud forms, we have recorded some very interesting radar echoes from the aurora at a wave-length of 23 cm. The observations have been made with a high-power *L*-band radar possessing the following system parameters :

Radar frequency = 1,306 Mc./s.
Pulse recurrence frequency = 100 p.p.s.
Pulse-length = 5 μsec.
Power = 2 MW.
Aerial rotation = 4 r.p.m.
Horizontal beam-width = 0·7° (between half power points)
Vertical polar diagram = cosec.² (lobe maximum at 1·6°)
Polarization = horizontal
Aerial gain = 10,000

During the two years of observations with this equipment the following three sets of auroral echo records have been secured :

Date	Duration of radar aurora
July 15, 1959	15 50–17.15 U.T.
April 1, 1960	14 00–17 50 U.T.
October 6, 1960	16.50–18.25 U.T.

The appearance of the radar aurora as seen on the plan position indicator at 17.43 U.T. of October 6 1960, is shown in Fig. 1. This record shows those essential features of the fully developed aurora which have been substantially reproduced on the occasions listed above, namely, the presence of two auroral arcs, the ranges of the extremities of which lie between 600 km. and 1,200 km., the azimuthal position of the arcs and their lateral extent. The rapidly changing form of the aurora is rendered vividly apparent by the method of time-lapse photography whereby photographs of the plan position indicator are taken so that successive scans of the aerial occupy consecutive frames of a ciné film. Projection of such a film through an ordinary ciné projector running at 16 frames per sec. gives a time compression factor of 240 ; this technique permits the movements of the auroral forms to be clearly observed and measured. The real or apparent motions of the scattering electron clouds within the auroral arcs will be discussed elsewhere ; but it is of interest to note that there is a marked tendency for the western limb to show a preponderance of 'receding' clouds.

An interesting feature of the observations of July 15, 1959, was that this auroral record followed a series of plan position indicator photographs taken at sunrise (03.59 U.T.) on the morning of July 14 when the disturbed state of the Sun was made manifest by the greatly enhanced solar noise signal received by the radar. These two direct observations, on the one radar equipment, of the solar disturbance and the subsequent aurora set a time-interval between the two events of 36 hr. ; this figure has to be increased to 36½ hr. when the time of first onset of the solar disturbance, as observed by S.I.D.s in the eastern hemisphere, is allowed for. (Solar flare, importance 3+, 03.25 U.T., 14.7.59.) Such a time-interval would correspond to an average velocity of the particles emitted from the Sun of the order of 1,133 km./sec.

Examination of the advices of magnetic data issued by the Eskdalemuir Observatory shows that

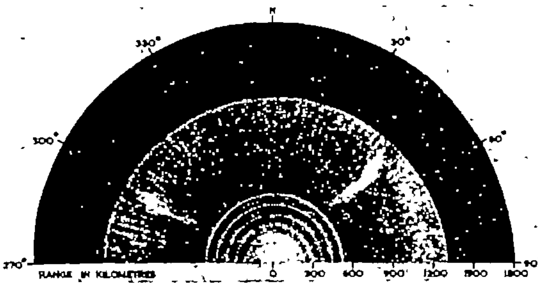

Fig. 1. Radar echoes from the aurora on 1,300 Mc./s. (17·43 hr., October 6, 1960)

the usual disturbance to the H-field there is of the order of 100–300γ. For April 1, 1960, however, disturbances of 2,600γ were recorded, while the magnetic storm of July 15, 1959, was even more violent in that disturbances of 4,100γ were observed. Survey of the records since 1949 shows that there have been only 21 occasions during the past 11 years when the disturbance in the H-field has exceeded 1,000γ; if these events are listed in order of diminishing intensity, then the first six entries appear as follows:

LIST OF MAGNETIC DISTURBANCES (1949–60)

Order	Year	Date	H-disturbance (γ)	Radar aurora (1,300 Mc./s.)
1	1959	July 15	4,103	Observed
2	1958	February 11	2,762	Equipment not in use
3	1960	April 1	2,592	Observed
4	1957	January 22	2,340	Equipment not in use
5	1957	September 4	2,300	Equipment not in use
6	1960	October 6	2,280	Observed

It is clear from this correlation between the occurrence of the radar auroræ reported in this article and the intensity of the magnetic disturbances as recorded at Eskdalemuir that only the more violent solar eruptions are likely to produce disturbances in the Earth's ionosphere of intensity sufficient to yield auroral echoes on a radar operating at so high a frequency as 1,300 Mc./s.; such events are likely to be comparatively rare.

The nature and spatial distribution of the radar echoes obtained at very high frequency during auroral activity have been studied by a number of workers; these investigations have clearly established the existence of aspect sensitivity in auroral radio reflexions. Chapman[1] has described the geometry of the reflexion process on the assumption that the reflecting 'rays' of the aurora are lying along the lines of force of the geomagnetic field, this latter being represented by the field of the equivalent centred dipole. Booker[2] has sought to reduce the degree of aspect sensitivity suggested by Chapman by a theory of scattering which postulates non-isotropic irregularities in the density of electrons in the aurora, such regions having their major axes parallel to the Earth's field. Booker[3] has applied his theory to the scattering of radio waves at frequencies up to 100 Mc./s., and in his treatment he assumes that the electron density in the aurora is increased only by a factor 10 over that normally obtaining in the E-layer. Fricker *et al.*[4] have obtained substantial agreement between the positions of auroral echoes observed at 400 Mc./s. and the areas of reflexion derived by approximate specular reflexion from columns of electrons lying along the Earth's field lines; in this analysis the actual Earth's field appropriate to the location of their radar stations was employed as distinct from the field inferred from the equivalent dipole approximation. The same process of specular reflexion was suggested by Peterson[5] to be the explanation of certain middle latitude echoes of auroral type, and Watkins[6] has shown that specular reflexion is closely obeyed in the case of the auroral echoes obtained at Jodrell Bank on wave-lengths of 4 m. and 8 m. Similar results have been reported by Harrison[7] from the International Geophysical Year auroral studies at Halley Bay.

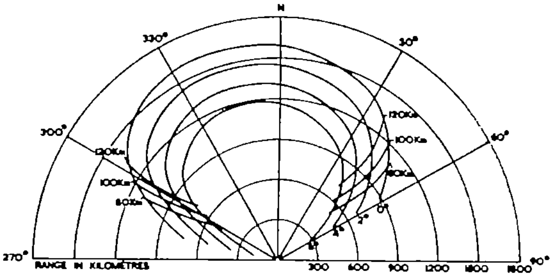

Fig. 2. Polar map for Bushy Hill, Essex, showing loci of feet of perpendiculars on the lines of force of the Earth's magnetic field

It is the high resolving power in both range and azimuth of the 1,300 Mc./s. radar equipment that has permitted the form of the aurora to be photographed with the precision shown in Fig. 1. The detail contained in this record allows the theory of radio reflexion from field-aligned regions of electron concentration to be tested and the stringency of the specular reflexion condition assessed in the case of a wave-length of 23 cm.

In Fig. 2 are shown the loci of the feet of the perpendiculars drawn from the radar site at Bushy Hill, Essex, to the lines of force of the Earth's field; each curve corresponds to a given angle of elevation of the rays from the radar. The magnetic data from which these curves have been calculated was taken from the Admiralty charts of isogonics and isoclinics (January 1960). Also included in Fig. 2 are the curves of constant height corrected for atmospheric refraction.

It will be seen that the area of Fig. 2 included between the 80 km. and 100 km. height curves and lying between the 0° and 6° elevation loci corresponds very closely indeed to the auroral arcs of Fig. 1. Now the beam-width of the radar in the horizontal plane is 0·7° while the angle subtended by a tangential element of the auroral arc at 1,000 km. is 5°. It is clear, therefore, that the azimuthal extent of the auroral form of Fig. 1 is not attributable to the beam-width of the radar. The azimuthal location of a height curve as plotted on Fig. 2 is quite sensitive to the height parameter and so a possible interpretation of Fig. 1 in the light of Fig. 2 would be that the auroral reflexions commence in the region of the 80-km. level of the ionosphere and extend upwards to an approximate height of 105 km. Alternatively, the azimuthal spread of the auroral arc could be regarded as arising from rays incident at angles between 90 ± ½° on the auroral columns of ionization located at a height of 90 km. It is probable that both the aspect and altitude effects play their parts in forming the auroral patterns of Fig. 1, but the present microwave experiments clearly show that the departure from the 90° reflexion condition is less than ± ½°, and that the region of the ionosphere where the auroral reflexions take place is of the order of 20 km. thick.

The mean height of the auroral layer as deduced from Fig. 1 by measurement of the azimuthal spread of the two auroral arcs, that is, 90 km., is lower than the value suggested by other workers operating at much lower radio frequencies. The evidence given above suggests that an auroral disturbance of sufficient intensity to be observed at 1,300 Mc./s. will only occur as an aftermath of a very vigorous solar upheaval and in association with extreme magnetic storms. In these circumstances it might be expected that the solar emission would contain a proportion of more energetic primary particles capable of producing ionization at lower levels of the ionosphere and also of yielding auroral displays at lower latitudes. This southward movement of the aurora during the growth of intense magnetic storms has been discussed by Störmer[8] and has received confirmation in recent International Geophysical Year work[9]. It will be noted, however, that the kinetic energy of the solar particles at the moment of penetration into the Earth's atmosphere, if taken as that corresponding to the average velocity of transit from the Sun to the Earth, that is, 1,133 km./sec. as given above, would scarcely seem adequate to produce the observed microwave auroral effects at so low an altitude as 80–100 km. ; it is probable that account must also be taken of the flux density of the incident particles.

The fact that the aurora of Fig. 1 was not observed in a northerly direction from the radar is a consequence of the shape of the vertical radiation diagram of the aerial, which extends from 0·2° to 6°, these elevations corresponding to 10 decibels of power reduction relative to that of the lobe maximum located at 1·6°. This figure of 1·6°, when associated with the observed range of 1,000 km. for the position of the echo of maximum amplitude, gives a height of 98 km. for this particular reflexion point.

The signal/noise ratio measured for this echo was 1·3/1 ; since the receiver noise-level was 4×10^{-15} watt, it may be deduced that the equivalent radar cross-section of a region of the aurora which yields a single pulse point on the plan position indicator is 1·4 sq. m. By making reasonable assumptions as to the volume of the ionosphere contributing to this signal the echoing area per unit volume of the auroral scattering region may be estimated as of the order of 10^{-9} m.$^{-1}$.

If incoherent scattering by electrons is responsible for this reflexion of microwave energy, then, taking the Thomson cross-section of a single electron as of the order of 10^{-30} m.^{2} would lead to a density of electrons of 10^{14} per c.c. On the other hand the electron density corresponding to normal critical reflexion for a frequency of 1,300 Mc./s. would be 2×10^{16} electrons per c.c. It seems clear that neither of these mechanisms can be responsible for the auroræ observed at 1,306 Mc./s. and that a theory of scattering such as has been developed by Booker (*loc. cit.*) must be applied.

We wish to thank our colleagues of the Radar and Photographic Groups for their assistance with the observational programme and the Director of Eskdalemuir Observatory for supplying the magnetic data.

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A VERSATILE METHOD FOR SHORT-TERM SPECTRUM ANALYSIS IN 'REAL-TIME'

By J. S. GILL

Joint Speech Research Unit, Eastcote, Middlesex

THE variations with time of the short-term energy spectrum of signals such as speech, music, singing, etc., can be measured with a bank of electrical band-pass filters and rectifiers. This leads to the type of display in three dimensions (time, frequency, intensity) commonly known as a sound spectrogram. Spectrographic patterns have proved of great value in the study of complicated audio frequency signals, especially human speech. However, this method becomes impractical when large numbers of filters are required. For example, at least 160 filters would be needed to provide a reasonable display when analysing a band 4 kc./s. wide with filters 50 c./s. wide. A choice of analysis band-width is also often required, and this is particularly difficult to arrange on a large filter-bank.

The analysis can be performed in a much more economical and flexible manner by recording the signal and then analysing by repeatedly playing this recording through a single filter. The signal is swept slowly across the pass-band of this filter by means of a frequency-changing process[1]. (Any filter 'remembers' its immediate past history, and so it is not possible to analyse merely by sweeping the single filter rapidly across the frequency band occupied by the original signal[2].) The output of this filter is recorded on a surface, usually a sheet of electro-sensitive paper, which rotates synchronously with the recorded signal, thus producing a sound spectrogram. The time required for a complete analysis is at least:

$$T = t + \frac{Nt}{k}$$

where T = total time required, t = original duration of recorded signal, N = required number of separate analyses, k = play-back speed/recording speed. In most existing instruments of this type T/t is about 100.

There are many calls, particularly in the study of speech, for a versatile spectrum analyser the output of which unfolds as the speech proceeds in time, that is, operates in 'real-time'. Although a filter-bank can be used for this purpose, detailed analysis by this method is rather impractical, due to the large number of filters that are needed. The single filter method outlined above cannot provide 'real-time' operation, however large k may be, because recording and analysis do not proceed simultaneously. In the method to be described these two processes of recording and analysis are interleaved, as shown in Fig. 1, to enable a single analysis filter to be used for determination, in 'real-time', of the short-term spectral properties of a signal.

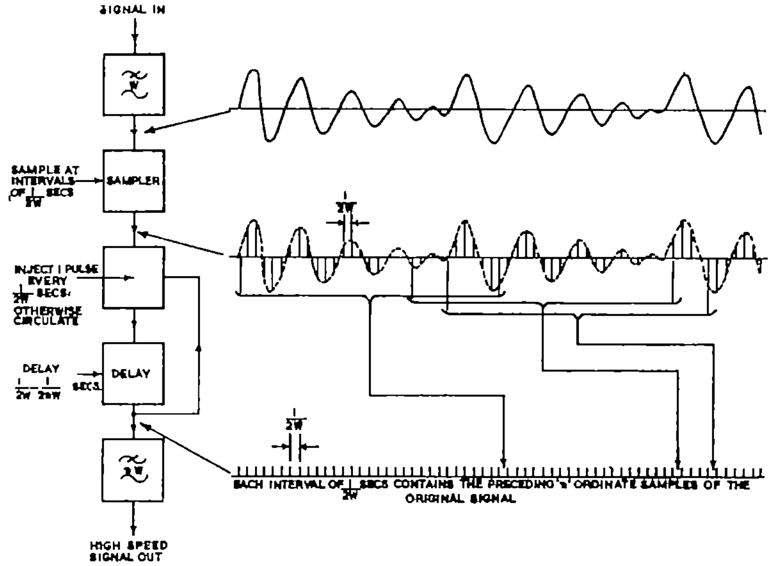
SIGNAL IN
SAMPLE AT INTERVALS OF $\frac{1}{2W}$ SECS
SAMPLER
INJECT 1 PULSE EVERY $\frac{1}{2W}$ SECS, OTHERWISE CIRCULATE
DELAY $\frac{1}{2W}-\frac{1}{2nW}$ SECS
DELAY
$\frac{1}{2W}$
$\frac{1}{2nW}$
EACH INTERVAL OF $\frac{1}{2W}$ SECS CONTAINS THE PRECEDING 'n' ORDINATE SAMPLES OF THE ORIGINAL SIGNAL
HIGH SPEED SIGNAL OUT

Fig. 1. High-speed repetition of the immediate past of a signal by means of a circulating store

The signal to be analysed is restricted to the frequency band 0–W c./s. and ordinate samples taken at $1/2W$ sec. intervals are injected into a circulating memory of length $1/2W - 1/2nW$ sec. which always contains the last $n-1$ signal samples. These samples are now spaced at $1/2nW$ sec. intervals, and during each $1/2W$ sec. interval the entire contents of the memory, namely, the past $n/2W$ sec. of the original signal, circulate at n times their original rate. Each ordinate sample is available during n successive circulations and can therefore contribute to n separate analyses, which are performed by a single analysis filter preceded by a frequency-changer.

The high-speed signal fed to this filter has discontinuities at intervals at $1/2W$ sec. The effects of these must be excluded from, or at least minimized in, the final display. This is possible provided that the majority of the impulse response of the analysis filter is contained within $1/4W$ sec. These unwanted transients can then be suppressed by inhibiting the analysis filter output during the appropriate half of each $1/2W$ sec. interval. The frequency-time plane is mapped as a sequence of strips, each of effective length $n/4W$ sec. The actual time required for each strip is $1/2W$ sec., and so it is possible to produce a spectrographic display comparable to that provided by a parallel filter-bank of $n/2$ filters.

It has been shown that a gaussian shape of filter characteristic gives the smallest uncertainty product[3] (time resolution × frequency resolution) and so this characteristic was chosen for the analysis process. The impulse response of such a filter is not of finite duration, and so the effects of the $1/2W$ spaced transients cannot be completely suppressed. In practice the analyser output is inhibited during the intervals in which these disturbances exceed one-tenth of their maximum amplitude. The requirements for the circulating memory are, for an idealized gaussian filter:

$$ft \simeq 1\cdot14$$

where f = bandwidth at the $\frac{1}{2}$ power points, t = duration of impulse response at a tenth of maximum amplitude. So the unwanted transients can be suppressed and the frequency-time plane mapped when:

$$\frac{1}{2} \times \frac{1}{2W} \times \frac{1}{2W\tau} > \frac{1\cdot14}{f}$$

where τ = digit spacing within the memory. Hence, $\tau < f/9W^2$.

A magnetic or dielectric drum can be used for storage when the sampling-rate (and hence the required rate of rotation) is fairly low—say, less than 500 per sec. No wholly analogue method has yet been found that is suitable for storage and high-speed repetition of wider band-width signals such as speech. For this purpose the only satisfactory solution,[4] at

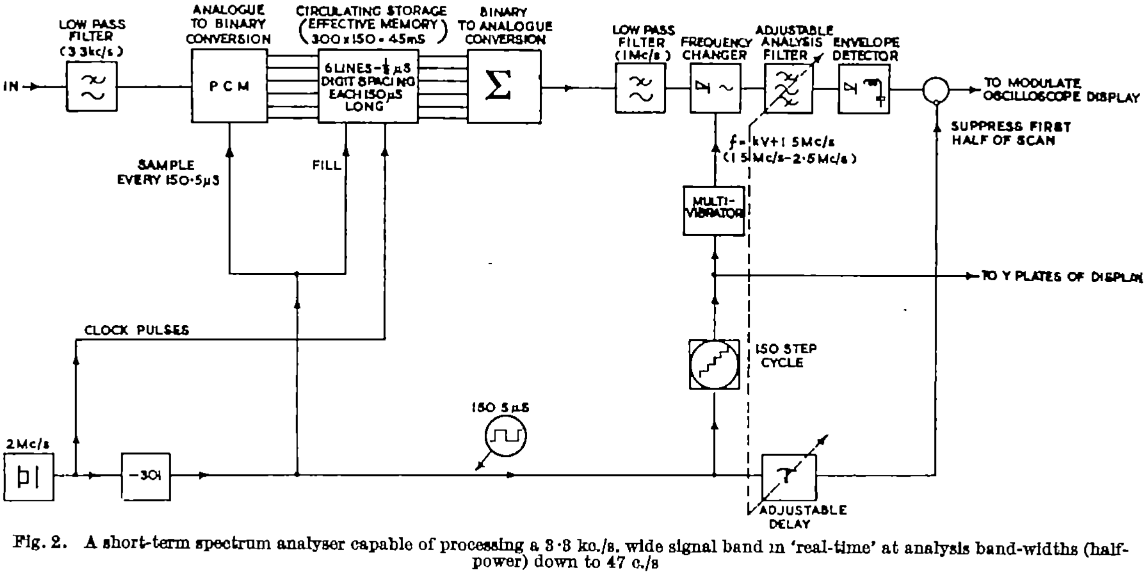
IN
LOW PASS FILTER (3·3kc/s)
ANALOGUE TO BINARY CONVERSION
PCM
CIRCULATING STORAGE (EFFECTIVE MEMORY 300×150 = 45mS)
6 LINES – ½ μS DIGIT SPACING EACH 150μS LONG
BINARY TO ANALOGUE CONVERSION
Σ
SAMPLE EVERY 150·5μS
FILL
CLOCK PULSES
2Mc/s
÷301
150 5μS
LOW PASS FILTER (1Mc/s)
FREQUENCY CHANGER
ADJUSTABLE ANALYSIS FILTER
ENVELOPE DETECTOR
TO MODULATE OSCILLOSCOPE DISPLAY
SUPPRESS FIRST HALF OF SCAN
f = kV+1 5Mc/s (1 5Mc/s–2·5Mc/s)
MULTI-VIBRATOR
TO Y PLATES OF DISPLAY
150 STEP CYCLE
ADJUSTABLE DELAY

Fig. 2. A short-term spectrum analyser capable of processing a 3·3 kc./s. wide signal band in 'real-time' at analysis band-widths (half-power) down to 47 c./s

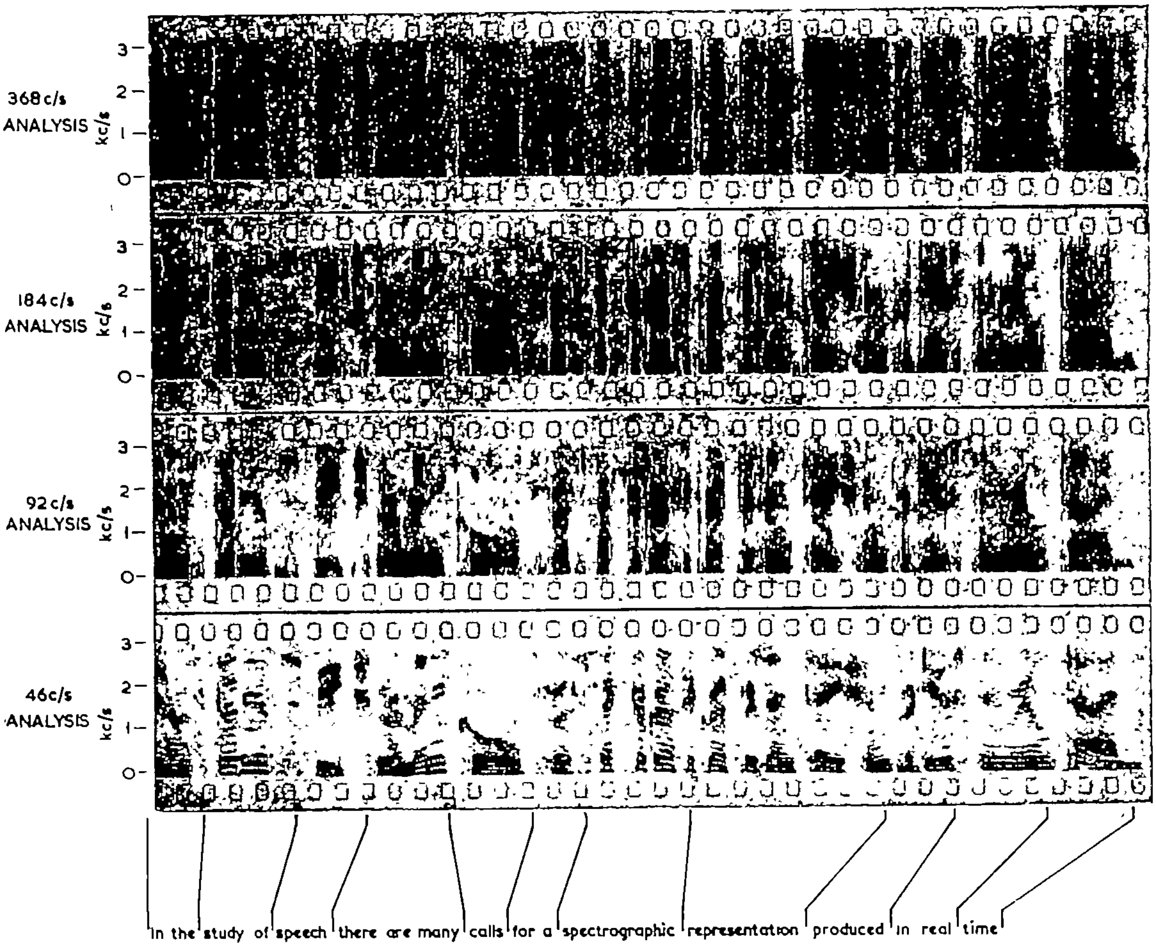

Fig. 3 Continuous spectrograms produced by the analyser shown on Fig. 2

present, is to express each ordinate sample as a set of *n* binary digits (that is, digitally encode the signal by means of pulse code modulation) and to store and repeat the signal in this binary form.

'Real-time' spectrum analysis of a 3·3 kc./s. wide band at analysis band-widths down to about 50 c./s. has been achieved by the method outlined in Fig. 2. The signal is sampled at 150·5-µsec. intervals and encoded into 6 binary digits/sample by a pulse code modulator, the output of which takes the form of six streams of binary digits. Each one of these streams feeds its own circulating store into which these digits are packed at ¼ µsec. spacing. The stores consist of magnetostrictive-acoustical delay lines of length 150 µsec. which use wire for the delay medium[4]. The six high-speed streams are decoded into a sequence of high-speed replicas of the immediate past 45 m.sec. of the signal, which are presented for analysis at 300 times their original rate. This high-speed signal is stepped at 150·5 µsec. intervals across the pass-band of a fixed analysis filter and the resulting display is of the form shown in Fig. 3.

The useful dynamic range of the analysis cannot, at any time, exceed that of the orginal binary digital encoder. In general, the range will be somewhat less than this and dependent on the structure of the signal. The appearance of the spectrograms shown on Fig. 3, which were produced by this continuous analyser, suggests that six bits/sample is probably sufficient for most work on speech. If necessary, the dynamic range can be increased by digitally encoding the original signal into a larger number of bits/sample.

This new instrument provides, for the first time, a continuous analysis of resolution sufficient to show both the gross spectral envelope of the speech (determined by the resonances of the vocal tract) and the fine detail associated with the exciting source (for example, the harmonic structure that characterizes the pitch of voiced sounds). This may materially assist work on voice training and speech correction[5]. The method can provide a suitable basis for excitation and system function[6] estimation in analysis–synthesis systems of telephony. Other more speculative applications such as phonetic transcription[7,8] and sound coding can be visualized.

I wish to thank Dr. J. Swaffield, head of the Joint Speech Research Unit, for his support and encouragement, P. F. Leach for much of the detailed design and D. Calvert for construction of the instrument.

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NUCLEIC ACIDS IN DEUTERIUM OXIDE SOLUTION

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AND
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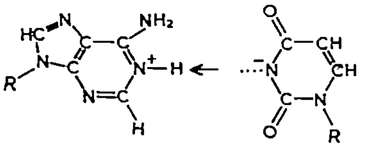
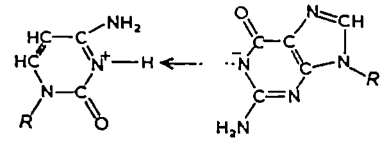
An Infra-red Criterion of the Base Pairing in Nucleic Acid Structure

IN the course of work on the infra-red spectra of nucleosides[1], we have found that, from a 1-mole : 1-mole aqueous mixture of adenosine and uridine, a complex crystal is obtainable, the 6μ-region spectrum of which is greatly different from the sum of the spectra of the adenosine and uridine crystals (Fig. 1, *A*). In the spectrum of the complex crystal a strong band is observed at 1,700 cm.$^{-1}$ which is not observed in the adenosine spectrum or in the uridine spectrum. In the spectrum of adenosine hydrochloride, a strong band is observed at 1,693 cm.$^{-1}$ (not far from 1,700 cm.$^{-1}$), which is attributable[1] to a stretching vibration of the C = N bonds of the purine ring where the π-electrons are localized at the C = N bonds to a greater extent than in the purine ring of free adenosine. These facts are explained by considering that there is a base–base interaction between the adenine and uracil residues in the complex crystal, and that in this interaction there takes place a proton transfer from the uracil residue to the adenine residue such as :


(1)

which causes a π-electron localization[1] in the C = N bonds of the adenine residue, giving rise to the strong band at 1,700 cm.$^{-1}$.

From a 1-mole : 1-mole mixture of aqueous solutions of cytidine and guanosine, a complex crystal was obtained which gives a strong band at 1,720 cm.$^{-1}$ (see Fig. 1, *B*). Neither in the spectrum of cytidine crystal nor in that of guanosine crystal are strong bands observed in the vicinity of 1,720 cm.$^{-1}$, but in the spectrum of cytidine hydrochloride crystal a strong band is observed at 1,725 cm.$^{-1}$. The latter band can be assigned to the stretching vibration of the C=O bond of the cytosine residue which has acquired a proton. Therefore, the 1,720 cm.$^{-1}$ band of the cytidine–guanosine complex is attributable to a proton-transferred structure :


(2)

which may be formed by the base-base interaction of the two nucleosides.

Similar strong bands at about 1,710 cm.$^{-1}$ are observed for the complex of cytidine and uridine and for that of adenosine and guanosine, but not for the complex of adenosine and cytidine nor for that of guanosine and uridine[1].

All the above facts suggest that the specific pairings of cytosine–guanine and adenine–uracil generally postulated in nucleic acid structures[2] involve proton transfer from uracil or guanine to adenine or cytosine, and that such pairings cause strong absorptions in the vicinity of 1,710 cm.$^{-1}$. Therefore, the appearance of the strong band at 1,710 cm.$^{-1}$ may be used as a criterion of the existence of the specific base-pairing.

We have observed 6μ-region spectra of some nucleic acids and nucleoproteins in their solid films[3]. Some of the results are given graphically in Fig. 2. The sodium salt of deoxyribonucleic acid from calf thymus placed in air of relative humidity 75 per cent shows a strong band at 1,708 cm.$^{-1}$, but in dry air a much weaker band here. This fact may be taken as indicating that the specific base–base

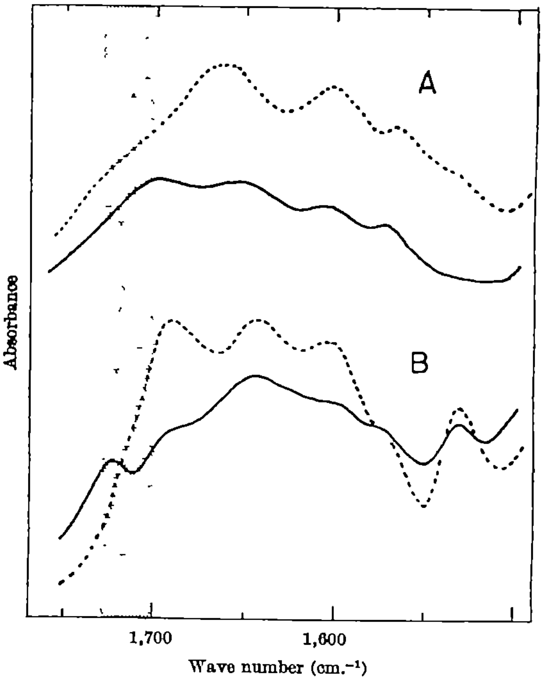


Fig. 1. *A*, infra-red spectrum of a complex (1 mole : 1 mole) of adenosine and uridine (——), and the sum of the spectra of adenosine and uridine (– – –); *B*, infra-red spectrum of a complex (1 mole : 1 mole) of cytidine and guanosine (——), and the sum of the spectra of cytidine and guanosine (– – –)

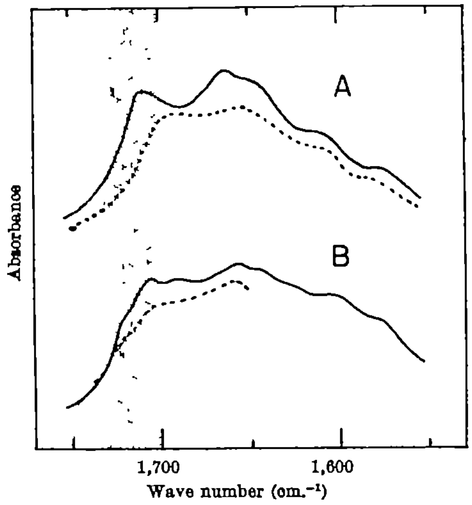


Fig. 2. *A*, Infra-red spectra of sodium salt of deoxyribonucleic acid film (from calf thymus) in air of relative humidity 75 per cent (——), and of the same film in dry air (- - -); *B*, infra-red spectra of a native sodium salt of ribonucleic acid film from microsomal particles of *E. coli* (——), and a film obtained from a solution of the sodium salt of ribonucleic acid heated at 100° C. for 10 min. (- - -). Both are observed at 75 per cent relative humidity

interaction is much weaker in the dry film than in the wet film. This is in accordance with what was concluded from other evidence[4,5]. The sodium salt of ribonucleic acid prepared from *E. coli* microsomal particles by the phenol method shows a moderately strong band at 1,705 cm.$^{-1}$ both in its dry and wet states. When it is heated at 100° C. for 10 min. or treated with ribonuclease, however, the 1,705-cm.$^{-1}$ band is observed with much weaker intensity, just as is expected.

A strong band appears at 1,710 cm.$^{-1}$ for the sodium salt of nucleohistone (deoxyribonucleic acid–histone) from calf thymus. This fact may be interpreted as indicating that there are numerous base pairings in deoxyribonucleic acid–histone, in accordance with the result of X-ray work[6]. For some samples of magnesium salt of microsomal particles from rat liver[7], only a weak band is observed at about 1,710 cm.$^{-1}$. This may indicate that, in such microsomal particles, a part of the base residues is bound to the protein and not available for the base–base interaction.

We have observed 6μ-region spectra of various nucleic acids and nucleoproteins, not only in their solid films but also in their deuterium oxide solutions. Results of these observations and the interpretations on the basis of the criterion suggested here will be given in detail elsewhere.

We wish to express our thanks to Dr. M. Takanami for his kindness in supplying rat liver microsomal particles. This work was aided in part by a grant from the Scientific Research Fund of the Ministry of Education of Japan.

Parallelism of the Hypochromicity at 260 mμ and the Hyperchromicity at 1,685 cm.$^{-1}$ of Nucleic Acids in Deuterium Oxide Solution

In a previous communication[8], it was shown that the absorption band at about 1,685 cm.$^{-1}$ of nucleic acids (deoxyribonucleic and ribonucleic acids) in deuterium oxide solutions is attributable to the base residues which are involved in the specific base–base pairings. In order to obtain further information of the nature of this 1,685-cm.$^{-1}$ band, we have observed the 6μ-region spectra of deoxyribonucleic acid and ribonucleic acid in their deuterium oxide solutions at various temperatures.

Some of the results obtained are shown in Fig. 3. Here, the samples used are the sodium salt of calf thymus deoxyribonucleic acid and the sodium salt of ribonucleic acid from rat liver microsomal particles. These are dissolved (5–10 per cent) in a deuterium oxide solution which is 0·1 molar in potassium chloride. The deoxyribonucleic acid solution was heated to 93° C., and the ribonucleic acid solution to 70° C. After they were kept at these temperatures for 1–2 min., they were cooled and the spectral changes were examined for reversibility. As is seen in Fig. 3, the most conspicuous spectral change that occurs with the change of temperature of the solution is that of relative intensity of the 1,685 cm.$^{-1}$ band.

In Fig. 4, the reciprocal of the intensity of the 1,685 cm.$^{-1}$ band relative to that of the 1,665 cm.$^{-1}$ band is plotted against the temperature of the solution. In the deoxyribonucleic acid solution there is a sharp change (transition) at about 85° C., while in the ribonucleic acid solution the change occurs gradually from 30° C. to 70° C. The 'cooling curve' lies always somewhat higher than the 'heating curve'. This trend is more evident when the maximum temperature reached is higher or when the solution is

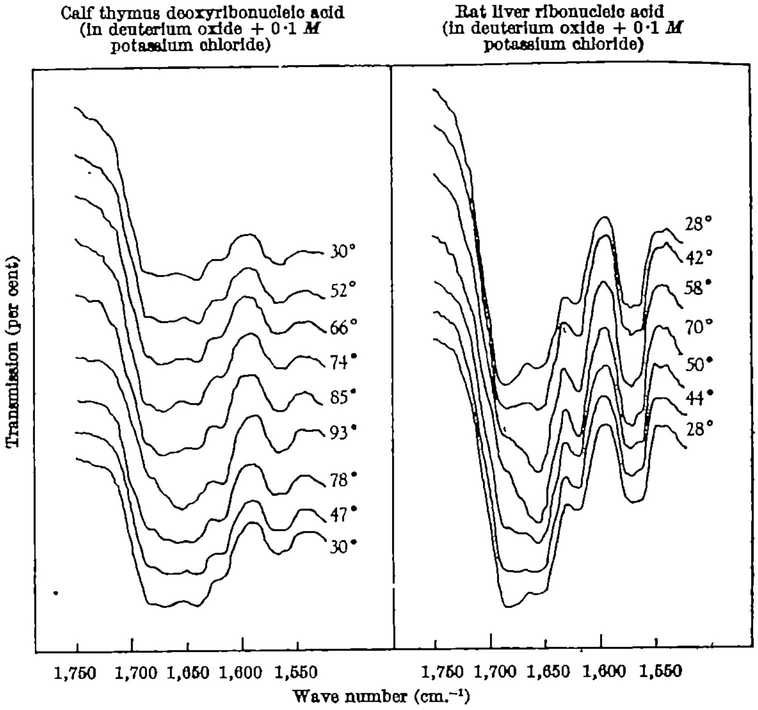


Fig. 3. Infra-red absorption spectra of deoxyribonucleic and ribonucleic acids in deuterium oxide solutions at various temperatures

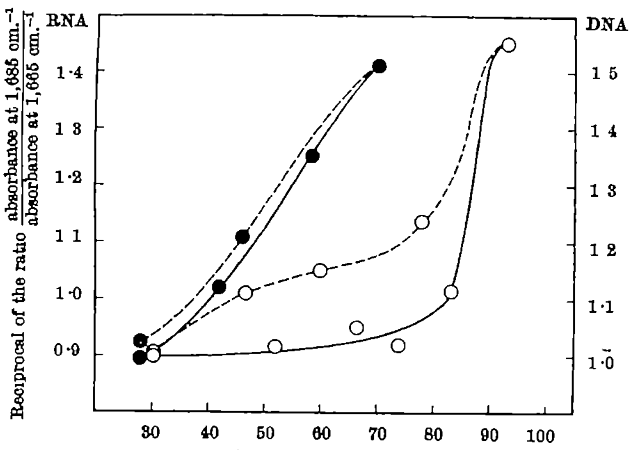


Fig. 4. The variation in the reciprocal of the relative intensity of the 1,685 cm.$^{-1}$ band as a function of temperature of the solution. — ○ —, Heating curve of deoxyribonucleic acid; -- ○ --, cooling curve of deoxyribonucleic acid; — ● —, heating curve of ribonucleic acid; -- ● --, cooling curve of ribonucleic acid

kept at the maximum temperature for a longer period.

All these features of the curves of reciprocal intensity at 1,685 cm.$^{-1}$ versus temperature of the solution are very similar to those of the corresponding curves of optical density at 260 mμ versus temperature of the solution given by Marmur and Doty[9] and by Doty, Boedtker, Fresco, Haselkorn and Litt[10]. Therefore, it is very probable that the change in the electronic structures of the base residues that causes the 1,685 cm.$^{-1}$ band (1,710 cm.$^{-1}$ band of the undeuterated base residues (preceding communication)) is directly correlated with the hypochromicity at 260 mμ.

We have also examined the relative intensity of the 1,685 cm.$^{-1}$ band at various temperatures of the yeast-soluble ribonucleic acid with sedimentation constant 4 Svedberg units. The reciprocal intensity–temperature profile of the soluble ribonucleic acid was found to be practically the same as that of rat liver microsomal ribonucleic acid in spite of the great difference in their molecular weights.

We wish to express our thanks to Dr. M. Takanami for the sample of rat liver microsomal ribonucleic acid and to Dr. S. Osawa for the sample of yeast-soluble ribonucleic acid. The expense of this research has been partly defrayed from grant-in-aid for scientific research from the Ministry of Education of Japan.

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GRADIENT OF CHOLINESTERASE ACTIVITY AND OF CHOLINE ACETYLASE ACTIVITY IN NERVE FIBRES

Gradient of Cholinesterase Activity

THERE are many indications that cholinesterase is synthesized in the cell bodies of neurons and carried down the axons towards the nerve endings (cf. Hebb[1]). The mechanism of the transport is unknown. It seems that information concerning the fate of the enzyme along the axonal path at various distances from the cell bodies and end organs might serve as one of the preliminary steps towards elucidation of this problem.

The nerves used for this kind of investigation should fulfil the following requirements : (1) the nerve trunk should be unbranched, so that at any level of the nerve all fibres are approximately equidistant from their terminal organs ; (2) it should be long enough to permit determinations of cholinesterase activity at several levels of the nerve.

The phrenic nerve of the dog satisfies approximately these conditions. The thin fascicles innervating intrathoric organs are easily separated from the main bundle to the diaphragm. The amount of protein per unit length was found to be the same at all levels of this bundle. The phrenic nerve has the additional advantage of being one of the most regularly discharging muscle nerves of the body. The regular alternation of periods of activity and of rest is likely to lead to a steady state between synthesis and utilization and, consequently, to constant amounts of enzyme at any given level of the nerve.

The phrenic nerves used were some 200–300 mm. long from the exit from the vertebral column to the level of branching near the diaphragm. They were removed from living anæsthetized animals, divided into three parts and cleaned of epineurium under the dissecting microscope.

Cholinesterase activity was determined by an ultra-microtitrimetric method of W. Niemierko (personal communication). Acetylthiocholine iodate was used as substrate, following McOsker and Daniel[2], and no separate determinations of true and pseudo-cholinesterase have been made so far. About 12 mgm. of fresh tissue were necessary for each determination, consisting of two parallel runs and one blank.

There are considerable differences in cholinesterase activity in various animals, and differences up to 30 per cent between the right and left nerve of the same animal.

Table 1 shows the cholinesterase activity in thirteen normal phrenic nerves at the upper, middle and low levels. In all nerves activity was slightly higher at the upper than at the lower level, and, in most cases, the middle level presented intermediary values. The differences between high and middle,

Table 1. CHOLINESTERASE ACTIVITY AT THREE LEVELS OF THE PHRENIC NERVE

Amount of acetylthiocholine split by 10 mgm. of fresh tissue in 2 hr. at 37° C., expressed in µl. of 0·1 *M* solution of the substrate

Dog	Upper	Middle	Lower
P.15—left	5 54	5·72	4·22
P.15—right	6·17	5·95	5 32
P.17—left	3 15	3·50	2·88
P.17—right	4·37	3 64	3·98
P.18—left	5 92	5 31	5·69
P.18—right	6·11	5 31	4·93
P. 1—left	4·39	4·15	3 15
P. 1—right	4 12	3 41	3 06
P. 2—left	4·12	3 45	2·82
P. 2—right	5 07	4 05	4 15
P. 9—right	5 14	5·28	4·62
P.10—right	7 08	6 68	6·71
P.19—right	6·38	5 62	5 31
Mean	5·20	4·77	4·37
Percentage of the lower level	119	109	100

and between high and low levels, are significant (*t*-test) at $P > 0{\cdot}99$. The difference between the middle and low levels is significant at $P > 0{\cdot}95$.

After section of the nerve, the amounts of cholinesterase and of choline acetylase[4] increase above the site of lesion. This increase is attributed by Hebb to the accumulation of the enzyme carried down from the perikaryon and prevented by the lesion from further displacement towards the periphery.

It was interesting, therefore, to see how the normal distribution of cholinesterase throughout the length of the nerve is altered after nerve section. This was done in the following experiments. Under aseptic conditions, the phrenic nerve near the diaphragm was sectioned and a portion of it removed and analysed for cholinesterase activity. The value obtained served to estimate the normal cholinesterase content of the nerve investigated at its low level. Then, after various time-intervals, the rest of the nerve was removed and cholinesterase determined at three levels of the proximal stump. The result of two experiments, 4 and 7 days after section, are shown in Fig. 1. The activity of the normal nerve was determined at the low level only; the probable normal values at higher levels are taken from percentages of Table 1. The results show that the increase of cholinesterase activity immediately above the site of section is accompanied, initially, by depletion of enzyme in the more proximal parts of the nerve.

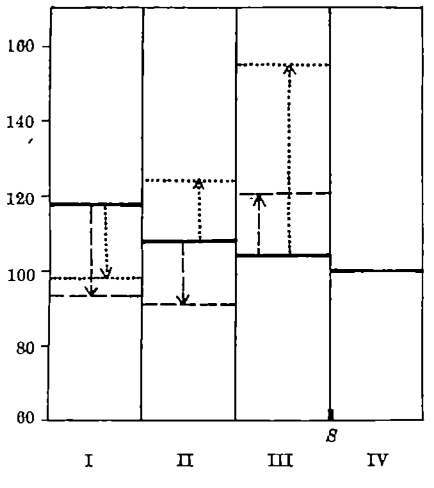

Fig 1. Changes of cholinesterase activity after section of the nerve at various levels of the proximal stump. I, II, III, IV represent portions of the nerve, the most proximal at the left. *S*, site of section. Ordinates: cholinesterase activity, as a percentage of that of the lowest part of normal nerve. Heavy line, normal nerve; broken line, 4 days after section; dotted line, 7 days after section

It should be noted that the phrenic is a very thin nerve and therefore rather long segments, of 60–100 mm., had to be taken in order to get enough tissue for determination of cholinesterase. In places where the differences of activity are likely to vary abruptly, as in the terminal part above the site of lesion, the use of long segments will tend to smooth out the real differences. A thick nerve, in which much shorter segments could be used, would therefore be more useful for exact determination of spatial re-distribution of the cholinesterase above the site of section, as was done by Hebb and Silver for choline acetylase (see following communication).

The re-distribution of cholinesterase activity in a sectioned nerve seems to indicate, besides the piling-up of the enzyme above the section, a slowing down of enzyme production by the perikarya in the initial period examined here. These results are in keeping with those of Schwarzacher[5] and Chacko and Cerf[6], who observed by histochemical methods a decrease of cholinesterase in perikarya during axon reaction, and they may be correlated with functional changes in respiratory discharge after section of the phrenic nerve described by Acheson, Lee and Morrison[7]. It should be noted that preliminary experiments in which the phrenic nerve was cut at the cervical level have failed to produce an increase of cholinesterase activity above the site of lesion.

As a result of these experiments, we conclude that, in the unbranched trunk of the phrenic nerve, there is a proximo-distal gradient of cholinesterase activity, the activity of the most proximal part exceeding by some 20 per cent that of the distal part of the nerve. Such proximal excess would suggest that the cholinesterase is metabolized not only at the nerve endings but also in the axon, although it does not constitute a direct proof of such utilization.

The changes of distribution of cholinesterase along the nerve after section seem to indicate that the transport of the enzyme stored in the nerve continues to be peripheral even at the time when its production in the cell bodies is slowed down or abolished.

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Gradient of Choline Acetylase Activity

IT was shown earlier[1], that after section of a cholinergic nerve (the cervical sympathetic of the sheep) choline acetylase rises much above the normal concentration in the proximal trunk while, coincidentally, it falls steeply in the distal trunk. Unlike true cholinesterase[2], lipolytic esterase[3] and substance P (ref. 4) which behave somewhat similarly, choline acetylase eventually disappears completely from the degenerating distal portion of the nerve. This has been regarded as evidence that all the axonal acetylase is derived from the perikaryon, whereas the other substances such as cholinesterase which do not disappear completely may be derived in part from extra-neuronal elements[5]. The increase of choline acetylase proximally fits in with the view that it is manufactured in the cell body, for, on the hypothesis that in the normal nerve the enzyme is moving steadily distally, section of the nerve would interrupt its progress and cause it to accumulate at that point. However, although it may be accepted that there is a flow or movement of materials from cell to periphery, a concept first formally stated by Weiss and Hiscoe[6] and supported by experiments with isotopically labelled compounds[7], it is by no means clear how the movement takes place.

The simplest idea is that there is a flow of dissolved axoplasmic constituents; but experiments in this laboratory[8] have shown that most of the axonal choline acetylase is firmly bound to structural elements; and these we believe to be part of the system of membranes that is continuous with the endoplasmic reticulum in the cell body. Thus, transport of the enzyme would appear to depend on movement of some part of the structural framework of the axon, possibly the growth of the whole axon. To some extent the experiments reported here support this view.

In these experiments the sciatic nerve of the goat was sectioned approximately 5 cm. below the fusion of its constituent spinal roots. It was established initially that the rise in choline acetylase in the proximal stump reached a maximum 5–7 days after operation. It was also found that the rise, estimated by comparison with the corresponding contralateral nerve, was entirely confined to the nerve trunk, there being no significant difference in enzyme concentration in either the motor or the mixed spinal roots of the nerve on the two sides. At these more central levels, the concentration on the side of operation was always within 20 per cent of the controls, a variation which is within the overall experimental error of the method of the assay for choline acetylase.

The finding that the rise was limited to the nerve trunk led us to investigate the gradient of activity in the region of the nerve immediately above the section. For this purpose the proximal part of the sectioned nerve was divided into five portions, 1 or 1·5 cm. in length; each portion was analysed separately and then compared with the corresponding piece of nerve from the contralateral sciatic. Choline acetylase activity in each was expressed in terms of the unit length of nerve, since variations in protein and water content of the operated nerve might obscure changes in the enzyme concentration if this were expressed in terms of weight of tissue.

From the results given in Table 1, it is clear that the rise in enzyme 7 days after operation is confined to the terminal 3 cm. of nerve. In fact, it is probable that it is confined to an even shorter length of nerve, since the region immediately above the section always contained some damaged nerve tissue. Similarly, the length between 2 and 3 cm. (called $P3$ in Table 1) may extend beyond the area of increased enzyme activity. We therefore estimate that at 7 days after operation the change is limited to about 2–2·5 cm. of nerve immediately proximal to the level of damage or section. Since at 4 days the length of nerve affected is shorter, and since the rise in enzyme begins to subside after 7 days, it is a fair inference that the figures given represent the maximum length of nerve in which higher enzyme activity occurs.

Table 1. CHANGE IN CHOLINE ACETYLASE IN THE SCIATIC NERVE OF THE GOAT 7 DAYS AFTER NERVE SECTION

Proximal stump (segments numbered from the level of section)	Length (cm.)	Fresh wt. (mgm.)	Dry wt. (mgm.)	γACh/cm./hr.	Per cent change in sectioned nerve
$P1$ Sectioned nerve	1·0	327	34	147	+ 84
$P1$ Control nerve	1·0	158	35	80	
$P2$ Sectioned nerve	1·0	304	47	113	+ 117
$P2$ Control nerve	1·0	163	29	52	
$P3$ Sectioned nerve	1·0	227	51	122	+ 90
$P3$ Control nerve	1·0	131	31	64	
$P4$ Sectioned nerve	1·5	235	38	59	No change
$P4$ Control nerve	1·5	230	44	59	
$P5$ Sectioned nerve	1·5	233	45	58	+ 18 (less then signif.)
$P5$ Control nerve	1·5	238	33	49	
Distal stump					
$D1$ Sectioned nerve	2·5	719	162	3	− 94
$D1$ Control nerve	2·5	559	123	56	

These findings, which are in general agreement with those of Lubińska *et al.* (see preceding communication) on the cholinesterase gradient in the sectioned phrenic nerve of the dog, seem to dispose of any possibility that the accumulation of enzyme in the proximal part of a sectioned nerve depends on its free diffusion downwards, since it is difficult to see how such a process could produce so sharply localized an increase in enzyme activity. We can, however, suggest one situation in which free diffusion is compatible with a terminal increase in enzyme: that is, if there is an increase in the space into which protein can diffuse. It might, for example, be imagined that section of an individual axon would permit its expansion terminally as the growing tip reached out beyond the Schwann cell tube. Cajal[9] has shown that the neuroma typically consists in part at least of extensive growth of such exploratory nerve tips. Some curve back and some go forwards, together forming a brush-like growth. On such a hypothesis, however, it is difficult to account for the length of nerve involved. It is likely that at the most only a few millimetres are involved in this sprouting and in the ballooning and branching of axons which is also seen at this time[6].

It seems to us that the accumulation of enzyme we have described is more likely to be associated with the precocious formation of synaptic vesicles in the sectioned axons, as has been reported for sectioned frog sciatic nerves[10]. This is the kind of change that would escape detection by the light microscope, and might well involve much greater lengths of nerve than would the grosser changes described by Cajal and others. It is possible that what happens is that the severed axons undergo morphological changes which make them comparable to pre-synaptic terminals, a region where there is also relatively high choline acetylase activity. It may be that in terminals of a normal nerve, for example, at the motor end-plate or at the sympathetic ganglion cell, this relatively greater concentration of choline acetylase is due not only to branching of the axons but also to expansion of the membrane system which ultimately gives rise to the synaptic vesicles, particles thought to contain both choline acetylase and its product acetylcholine[11]. We suggest, therefore, a similar branching and budding of membranes at the tips of severed axons.

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GLYCEROL IN *MYCOPLASMA MYCOIDES*

Unbalanced Growth of *Mycoplasma mycoides* caused by Glycerol Deficiency

MYCOPLASMA MYCOIDES var. *mycoides*, the agent of contagious bovine pleuropneumonia, requires glycerol as an essential nutrient[1]. The concentration required for maximum turbidity increases from about 5 μgm./ml. in static cultures to about 50 μgm./ml. in cultures rotated mechanically to give a moderate degree of aeration. Glycerol is rapidly oxidized by cell-suspensions, probably by a flavoprotein-catalysed oxidation of α-glycero-phosphate[2]. If this is a pathway which is essentially irreversible, then the need for exogenous glycerol for lipid synthesis would be readily explained. The observation that cultures undergo rapid lysis after a period of growth in media containing limiting concentrations of glycerol suggested that glycerol deficiency might cause unbalanced growth.

It has been found that when fully viable cells are incubated in medium lacking glycerol, but adequate with respect to all other nutrients, they die at a rate of about 80–90 per cent per generation time. There was an early increase, followed by a decrease, in turbidity; an early loss of streaming birefringence; and an increase in the viscosity of the cultures, suggesting that lysis had occurred. Profound morphological changes were evident in electron micrographs taken during the course of incubation. In the early stages the cells became enlarged, and many had shrunken appendages. Later, there was a great preponderance of flattened ghost-like forms, and only a few electron-dense elements probably representing the remaining viable cells. 'Glycerol-less death' is prevented if another essential nutrient (uracil) is omitted or if chloramphenicol is added, and the onset of the morphological changes is retarded by the omission of uracil.

The hypothesis derived from these observations is that glycerol is needed for the synthesis of some surface structure, and that this synthesis is more sensitive to glycerol deficiency than is the synthesis of the cytoplasm. The greater requirement for glycerol under aerobic growth conditions is due to the greater rate of oxidation and consequent loss of glycerophosphate for synthetic purposes.

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A Polyglycerophosphate Compound from *Mycoplasma mycoides*

SOME preliminary work on the incorporation of glycerol labelled with carbon-14 by *Mycoplasma mycoides* var. *mycoides* has been carried out. The cells were grown in a partly defined medium (see preceding communication) to which 5–20 μc. of DL-glycerol-1-^{14}C was added per 100 ml. The total glycerol concentration was 4–20 μgm. per ml. The cultures were harvested after the end of the logarithmic phase, when glycerol had become limiting and streaming birefringence had largely disappeared. At this stage there is extensive conversion to non-viable ghost-like forms[1]. The cultures were centrifuged at 35,000*g* and the pellets extracted without preliminary washing. Although the main energy substrate was glucose, most of the glycerol was catabolized to lactate, and in none of the experiments was more than 8 per cent of the added isotope recovered in the pellet. Small amounts of non-dialysable activity found in the supernatant were presumably derived from cells which had lysed. Most of the carbon-14 in the pellet was present in substances which yielded glycerol or glycerophosphate compounds on hydrolysis, but about 10 per cent entered the ribose of ribonucleic acid where it was greatly diluted with unlabelled carbon.

In the first experiments the pellet was extracted with cold trichloroacetic acid, aqueous ethanol, and wet ethanol–ether according to a procedure used successfully for tracer studies with *E. coli*[2]. An unexpectedly large fraction (40–60 per cent) of the carbon-14 incorporated was not extracted by this method. Even after subsequent extraction with 5 per cent (w/v) trichloroacetic acid at 100° for 30 min., which removes the nucleic acids quantitatively from *M. mycoides*, a large amount of radioactivity remained in the residue. Alkaline hydrolysis of the latter released most of the remainder (33 per cent of the total incorporated) in the form of acid-soluble compounds. These included free glycerol and glycerophosphate, but at least three other compounds were present.

The results in Table 1 refer to an experiment in which the labelled cells were suspended in distilled water, frozen and thawed three times and then centrifuged at 26,000*g* for 15 min. It was convenient to include this step because about 80 per cent of the ribonucleic acid enters the supernatant fraction under these conditions. Only 9 per cent of the carbon-14 appeared in the supernatant, and nearly all this was accounted for as acid-soluble compounds and as ribonucleic acid ribose. After extraction with cold trichloroacetic acid, ethanol and ethanol–ether, the residue from the sedimentable fraction was washed with ether, dried and hydrolysed with 0·3 *N* potassium hydroxide at 37°. After 4 hr. and 24 hr., portions were cooled in ice, and protein, deoxyribonucleic acid and potassium perchlorate were precipitated by the addition of perchloric acid. The amount of carbon-14 rendered acid-soluble was not increased by prolonging the hydrolysis beyond 4 hr., but some activity remained unextracted. The acid-soluble products were taken up in 60 per cent (v/v) aqueous ethanol and applied to strips of Whatman 3 *MM* paper for ionophoresis in 0·05 *M* ammonium formate buffer, *p*H 3·6.

Table 1. DISTRIBUTION OF CARBON-14 IN FRACTIONS OF *Mycoplasma mycoides* GROWN IN THE PRESENCE OF DL-GLYCEROL-1-^{14}C

	c.p.m.	Per cent of total
Unfractionated suspension	142,000	(100)
Supernatant from frozen and thawed cells	13,000	9
Residue sedimented at 26,000*g* .		
Cold trichloroacetic acid soluble	800	1
Ethanol-soluble, water-soluble	5,800	4
Ethanol-soluble, ether-soluble	66,000	46
Acid-soluble fraction of potassium hydroxide digest	48,000	34
	133,600	94

Five heavily labelled bands were revealed by radioautography. These were well separated from each other and from the ribonucleotides, which were only feebly radioactive. The properties of the five concomitant compounds are summarized in Table 2. Ionophoresis was carried out by the hanging-strip method. The mobilities are corrected for electroendosmotic flow, but not for convection or other errors. Compounds I, II and III and their hydrolysis products were identified by chromatography in admixture with authentic carrier substances in at least three different systems.

Glycerol (G) and monoglycerophosphate (GP) were commercial products. α,α′-diglycerophosphate (GPG) was synthesized from DL-*iso*propylideneglycerol by the method of Fischer and Pfahler[3]. The phosphate esters were chromatographed as the ammonium salts and located with ammonium molybdate reagent[4]. Glycerol was located with silver nitrate. Incubation of compound *V* with the phosphatase gave a spot which migrated like GPG in four different systems. This suggested a structure (GPGP) in which an additional phosphate group was esterified to one of the remaining hydroxyls of GPG. Column 3 of Table 2 shows the changes in the relative densities of the bands on the radioautographs when the time of incubation in potassium hydroxide was increased from 4 to 24 hr. It suggests that compounds I, III and V, and perhaps II also, are formed by alkaline hydrolysis of IV. If so, one possible structure for compound IV was that consisting of three molecules of glycerol joined by two phosphate groups in diester linkage. This structure (GPGPG) is known to occur in the acidic phospholipid cardiolipin[5]. Cardiolipin was therefore prepared from fresh beef hearts[6] and saponified. The ethanol-insoluble potassium salt of the resulting polyglycerophosphate was converted to the ammonium salt and co-chromatographed with compound V in the following systems: water-saturated phenol (NH_3, HCN atmosphere), *iso*propanol/concentrated ammonia/water (70 : 6 : 30, v/v), and by ionophoresis at *p*H 3·5. In no case could separation of the radioactive material from the carrier be detected. No authentic sample of the dimer of glycerophosphate (GPGP) was available for comparison with compound V. It is of interest that several dinucleotides of analogous structure have been found among the products of alkaline hydrolysis of ribonucleic acid[7].

Table 2. PRODUCTS OF ALKALINE HYDROLYSIS OF THE RESIDUE FRACTION

No.	M_u*	Change 4–24 hr.†	Phosphatase‡	Acid hydrol.§	Diagnosis
I	0·00	Increase	—	—	G
II	1·14	Slight or nil	No change	G + GP	GPG
III	1·47	Increase	G	No change	$\alpha+\beta-$GP
IV	1·88	Decrease	No change	G + GP	GPGPG
V	2·30	Increase	GPG	GP (+G)	GPGP ?

* Anionic mobility at *p*H 3·6 relative to that of uridylic acid.
† See text.
‡ Products of hydrolysis with crude acid phosphatase at *p*H 5·5.
§ Products of 10 min. hydrolysis in *N* hydrochloric acid at 100°.

Consideration of the properties of the acidic phospholipids indicated that the procedure used might fail to extract them efficiently. It was found that, when the labelled cells were treated with 19 volumes of acetone at 0°, and then extracted with chloroform–methanol (2 : 1, v/v) at room temperature, no glycerophosphate compounds were present in alkaline hydrolysates of the residue. When the chloroform–methanol extracts were saponified, a large fraction of the carbon-14 was found in a substance which could not be separated from that obtained from cardiolipin when the two were co-chromatographed in the above three systems and also in *n*-butanol/acetic acid/water (5 : 2 : 3, v/v). Further work will be necessary to determine the nature of the substance extracted by chloroform–methanol, and its relationship to the process of 'glycerol-less death' (see preceding communication).

It is perhaps of more general interest that the ribonucleotide fraction, obtained from labelled cells which had been treated with cold trichloroacetic acid before extraction with chloroform–methanol, still contained radioactive substances migrating faster than uridylic acid at *p*H 3·5. These were not found if the cells had been precipitated with 4 volumes of ethanol at 77°, or with cold acetone as described above.

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LETTERS TO THE EDITORS

ASTROPHYSICS

The Dust Cloud about the Earth

The existence of a high concentration of interplanetary dust near the Earth has recently been demonstrated from rocket, satellite and space-probe observations of acoustical impact. Suspected by Beard[1], the concentration has been proved by me[2] and by Hibbs[3]. To a distance of approximately 10^5 km., the concentration falls off roughly as the inverse 1·4 power of the distance from the Earth's surface (Fig. 1). The impact-rates on randomly oriented surfaces include all particles greater than mass 10^{-9} gm. and are adjusted for the differing sensitivities of the equipment. Data are from six U.S. rocket firings; from satellites *Explorer I* (1958 Alpha), *Sputnik III* (1958 Delta) and *Vanguard III* (1959 Eta); and from the space probes *Explorer VI* (1959 Delta) and *Pioneer I*. At some distance greater than 10^5 km., the impact-rates approximate those expected from calculations on the zodiacal cloud; the maximum concentration with respect to the interplanetary medium is perhaps as high as 10^4.

Four possible explanations for the dust concentration cannot immediately be eliminated by simple physical arguments: (1) The electrostatic explosion of fragile particles by charges ($\sim$ 100 V.) induced in the radiation belts of the Earth. (2) Capture into temporary circumterrestrial orbits by high-charge, high-drag processes in the radiation belts of the Earth. (3) Gravitational capture into temporary circumterrestrial orbits in the four-body system of Earth–Moon–Sun and particle (Beard's prediction). (4) Expulsion of dust and droplets from the Moon by meteoritic crater formation into temporary cislunar orbits.

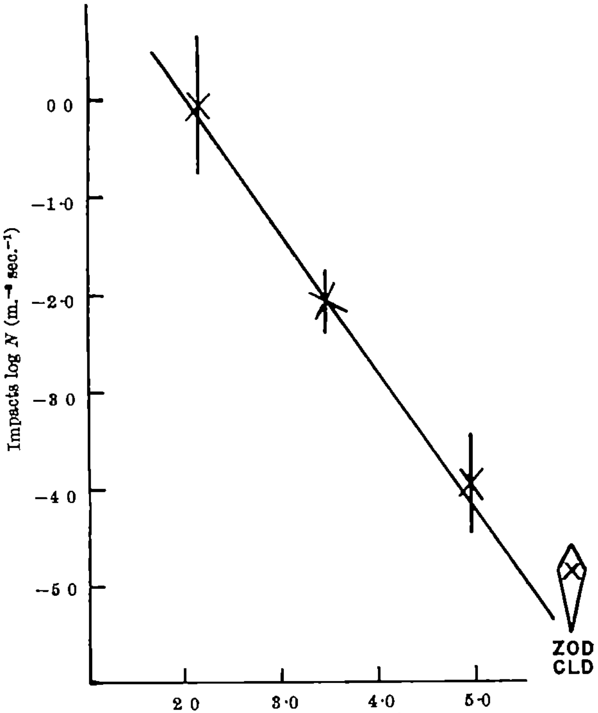

Fig. 1. Meteoritic impact (mass = 10^{-9} gm.) versus altitude from the Earth

Since there is no clear evidence that the Earth's radiation belts produce high electrostatic charges on particles, explanations (1) and (2) must be viewed sceptically until demonstrated. Gravitational capture of interplanetary particles by the Earth–Moon system appears to be a process of extremely small probability ($< 10^{-5}$?), but may be significant if the interplanetary orbits are nearly circular and of low inclination to the ecliptic. This type of motion has not been demonstrated. If capture orbits can persist for an unlikely 10^8 revolutions about the Earth, the small expected capture-rate might be adequate. I cannot verify Beard's theory of the three-body (Sun–Earth–particle) capture process, while Beard's predicted law of particle concentration near the Earth does not agree with the observed law. Hence explanation (3) shows doubtful promise.

Let us consider explanation (4). Meteoritic bodies of cometary and asteroidal origin certainly strike the Moon at high velocities, up to 73 km./sec., with perhaps a median value near 15–20 km./sec. Direct encounters from interplanetary orbits occur for the Moon at a rate less than 1/16 that for the Earth, this areal ratio being further reduced by the greater gravitational attraction of the Earth. The over-all rate may be approximately 2–3 per cent that for the Earth. No adequate theory yet predicts the high-velocity ejection component in crater-forming impacts. The solid material must consist almost entirely of dust and droplets, whatever the nature of the lunar surface; large ejection bodies, as suggested by Urey[4], should be extremely rare. Perhaps the dust ejected into cislunar orbits (2·4 < velocity < 3·4 km./sec.) may be a few per cent of the total mass striking the Moon, or of the order of 10^{-3} times the total meteoritic material directly striking the Earth. Since fine dust appears to be only a few per cent of the total solids striking the Earth directly, the two rates may be comparable within one or two orders of magnitude. From drag considerations, temporary circumterrestrial orbits for 10^{-9} gm. particles may possibly persist for 10^5–10^6 revolutions. If, indeed, the dust concentration factor is then greater than 10^3 within the Moon's orbit, the dust cloud about the Earth is semi-quantitatively explained. The higher concentration near the Earth would arise from convergence of the orbits and drag effects.

Thus a lunar origin for the dust seems fairly tenable. The hypothesis will predict a concentration in the plane of the Moon's orbit with generally a direct (eastwardly) motion of the particles. The dust cloud would be more comparable to the zodiacal cloud about the Sun than to the rings of Saturn (suggested by Hibbs). If this explanation is valid, the direct motion and distribution should be readily measurable in space experiments and the diurnal effects should be small for orbits close to the plane of the Moon's orbit.

Much more observational, analytical and theoretical research is required to clarify this surprising phenomenon.

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[1] Beard, D. B., *Astrophys. J.*, 129, 496 (1959).

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Ionospheric Disturbance at the Time of Cosmic Ray Increases

IN a recent communication Legrand and Helary[1] have reported the occurrence of an anomalous increase in the intensity of the total cosmic ray component at the Paris Cosmic Ray Survey Station on December 4, 1957. At about the same time an increase was also observed in the intensity of the nucleonic component at Thule, Greenland. This cosmic ray event was further described as being analogous to one which was observed at Paris on January 21, 1957 (ref. 2). It has been suggested that these events are manifestations of the precipitation into the Earth's atmosphere of high-energy protons which emanate from the disturbed Sun.

Such cosmic ray increases at ground-level are rare, and their relationship to terrestrial atmospheric disturbances is not yet well understood. It is of interest, therefore, to consider conditions obtaining in the Earth's atmosphere at the time of such cosmic ray enhancements.

The ionospheric measurements reported here have been taken from the data collected by high-frequency vertical-incidence sweep-frequency sounders at Resolute Bay, Baker Lake and Ottawa. These stations have geomagnetic latitudes of 82·9°, 73·7° and 56·9° respectively. The ionosondes are part of a net which has been operated in and near the auroral zone for a number of years by the Department of Transport for the Defence Research Telecommunications Establishment of the Defence Research Board, Ottawa. The sweep-frequency equipment is conventional (2–20 Mc./s.) and soundings are taken continually at 15-min. intervals. The solar and magnetic data were taken from the monthly bulletin, *Solar Geophysical Data*, Part *B*, published by the National Bureau of Standards, Boulder, Colorado.

The cosmic ray increase on December 4, 1957, occurred during a period of low solar activity. An examination of the data from the Canadian ionosondes showed that there were no unusual conditions in the early part of December. All the ionospheric measurements remained very close to the monthly median values for several days before and after the cosmic ray enhancement.

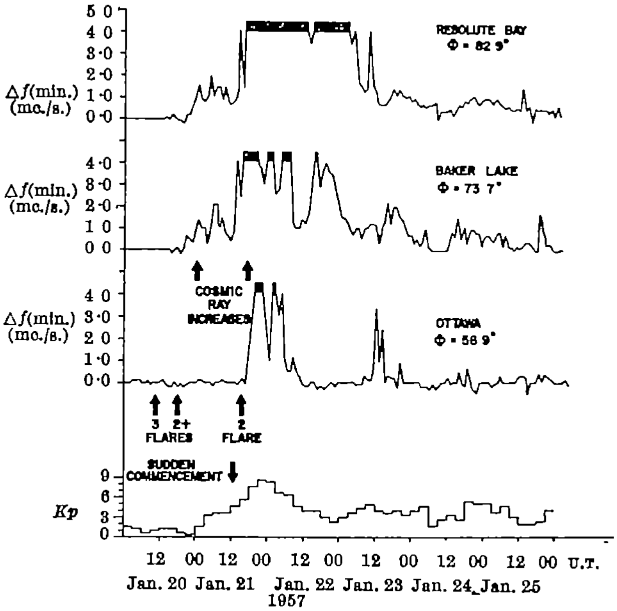

Fig. 1

The increase which was observed on January 21, 1957, occurred at a time of somewhat higher solar activity and the ionospheric conditions were quite different and considerably more complex. Both a geomagnetic storm and a polar-cap absorption event were observed during this period.

The polar-cap absorption event is now a familiar phenomenon, and, as a result of the International Geophysical Year work on radio-wave absorption at high latitudes, the gross features of these disturbances are fairly well known[3,4]. During a polar-cap absorption event the enhanced ionization in the lower ionosphere extends over the whole polar cap as far south as geomagnetic latitudes of approximately 60°. The disturbance lasts for several days and is much more intense when the ionosphere is sunlit. At such times high-frequency radio waves are completely absorbed, and very-high-frequency waves also suffer severe attenuation. It is generally believed that polar-cap absorption events are another manifestation of the precipitation of solar protons although these protons probably have somewhat lower energies (10–100 MeV.) than those responsible for the cosmic ray increase observed at the surface of the Earth[5].

Some of the measurements from three of the Canadian ionosondes which show the nature of the ionospheric disturbance in January 1957 are presented in Fig. 1. The three curves show the deviations from the monthly median values of the hourly values of *f*(min.) at Resolute Bay, Baker Lake and Ottawa. *f*(min.) is the lowest frequency reflected from the ionosphere. Since the lower frequencies are attenuated more strongly than the higher frequencies an increase in the value of *f*(min.) denotes an increase in absorption and hence an increase in the electron density in the lower ionosphere. The heavy horizontal bars at the tops of the curves represent intervals of blackout or complete absorption of the sounding transmission. The magnetic activity for the period (January 1957) is given by the histogram of *Kp* at the bottom of Fig. 1. An arrow denotes the time of the magnetic storm sudden commencement. Arrows marking the occurrence of flares are for the large solar eruptions which one might reasonably associate with the ionospheric effects. Times of maxima of cosmic ray increases have been taken from the report published by Legrand, Chanson and Bonpas[2].

It can be seen that the increase in Δf(min.) at the high-latitude stations on January 20 which marks the start of the polar-cap absorption began within a few hours of the first two large solar flares and was roughly coincident with the first cosmic ray increase. The second cosmic ray increase on January 21, which was slightly more intense than the first, followed the third flare by several hours. It has since been reported that the geomagnetic storm which began with the sudden commencement at 1230 on January 21 was accompanied by a Forbush decrease which began at Ottawa at 21 hr. (ref. 5).

Because of the timing, it seems likely that the magnetic storm and the Forbush decrease might be associated with the flares which occurred on January 20. One of these flares, it may be assumed, must have ejected particles with a wide range of energies if it were responsible for the observed ground-level increase of cosmic rays, the polar-cap absorption and the magnetic storm. Just what contribution was made to these events by particles from the third flare on January 21 is difficult to say since the polar-cap absorption event and the magnetic storm were already in progress at the time this flare occurred. It seems possible, however, that this last solar outburst produced only particles with very high energies since the polar-cap absorption event at Baker Lake was much reduced on January 22 and there was no second sudden commencement, nor was there any marked increase in the magnetic activity around January 23. Although the blackout at Ottawa occurred soon after the flare on January 21, it is not likely that these two events were related. It seems more probable that the particles producing the blackout at this station came from one of the flares on January 20 and were precipitated at the lower latitudes because of the distortion of the geomagnetic field at the time of the storm. This effect has been discussed by Freier, Ney and Winckler[6] in their description of the detection of solar cosmic rays below the normal cut-off rigidities appropriate to the latitude of Minneapolis on March 26, 1958.

The association of the foregoing events is of course highly speculative. It would seem, however, that although the cosmic ray increases in January and December of 1957 were in some respects analogous, they were associated with quite different ionospheric conditions. Further observations at the time of such cosmic ray increases are required to establish the nature of this relationship.

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RADIOPHYSICS

Irregular Fading of Satellite Transmissions

In a paper by Kent[1] it was stated that irregular fading of satellite transmissions on 40 Mc./s. from *Sputnik* 1 did not occur when the satellite was south of 50° N., and indeed was of very small amplitude on the average when the magnetic inclination at the sub-satellite point was less than 68°, the value at the observing station near Cambridge. Mawdsley[2] has suggested that this might be due not to the absence of irregularities to the south of Cambridge but to the southerly irregularities being incorrectly oriented to give appreciable power scattering to the receiver.

Some observations made at Slough on transmissions from the satellite *Explorer* 7 at a frequency of 20 Mc./s. are of relevance in this connexion. Although this satellite never reaches as far north as the latitude of Slough, irregular fluctuations are frequently found on the signal-strength records; they were of greater magnitude at night than during the hours of daylight. If a scintillation index is defined, as is commonly done, by the ratio of the standard deviation of the fluctuations to the mean signal amplitude, then the distribution of the mean index with magnetic inclination is as shown in Table 1; a separate index was calculated for each half-minute of record.

Table 1. Mean Scintillation Index in Certain Ranges of Magnetic Inclination of the Sub-satellite Point. Nig it-time Results Only, for the Period January–February 1960

Ranges of magnetic inclination (deg.)	No. of indices in each range	Mean index
50–56	23	0 10
56–58	23	0 11
58–60	30	0·14
60–62	59	0·14
62–64	98	0·18
64–66	206	0·17
66–68	121	0·24
68–70	13	0·27

The inclination at Slough is 67°; it can be seen that there are scintillations well to the south of Slough, although the index certainly tends to fall off as the satellite moves away from magnetic north. The decrease is not so sharp and does not continue to such a low level as that found by Kent on the 40 Mc./s. transmissions he observed.

The height of *Explorer* 7 lay in the range 550–800 km. during these observations. The positional information on which the work was based was derived from prediction data. Consequently the figures in Table 1 may be subject to errors of up to 2° in the magnetic inclination of the sub-satellite point; this is not sufficiently large to affect the conclusions given above.

This work forms part of the programme of the Radio Research Board. This communication is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

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PHYSICS

Random Fluctuations in a Nuclear Fission Reactor

The theory of the stochastic dynamics of a nuclear fission reactor has been formulated by Moore[1]. The present investigation is concerned with the measurement of time-dependent reactor parameters, using a spectral analysis technique and the formulation of both theory and experiment for the correlation analysis and measurement of spatially dependent reactor parameters.

The time-trace of the random fluctuations in neutron density in a nuclear reactor operating autonomously at low power has been recorded. From the direct recording of this 'noise' wave-form an amplitude spectrum and a spectral density-curve have been derived. Conclusions have been drawn concerning: (1) the effective, prompt neutron lifetime in the reactor; (2) the reactor transfer function.

Further experiments will be aimed at: (1) the measurement of the effective delayed neutron lifetimes, the temperature coefficient of reactivity and the study of possible 'neutron wave' effects; (2) the measurement of neutron age and other spatial parameters by a multi-channel correlation technique.

A block diagram of the recording apparatus is given in Fig. 1. The ionization chamber was placed centrally in the reactor core in a mean thermal neutron flux of 10^9 neutrons/cm.^{2}/sec. The inherent noise and long-term drift of the electronic circuitry were measured and minimized.

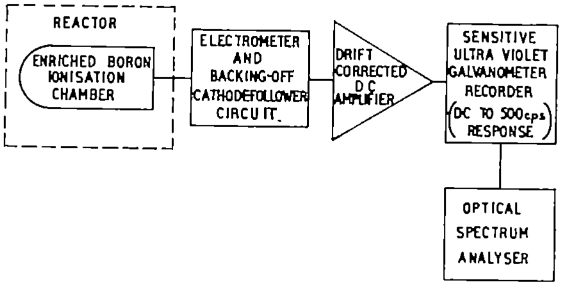
REACTOR
ENRICHED BORON IONISATION CHAMBER
ELECTROMETER AND BACKING-OFF CATHODEFOLLOWER CIRCUIT
DRIFT CORRECTED D.C. AMPLIFIER
SENSITIVE ULTRA VIOLET GALVANOMETER RECORDER (DC TO 500c.p.s. RESPONSE)
OPTICAL SPECTRUM ANALYSER

Fig. 1. Equipment for recording and analysis

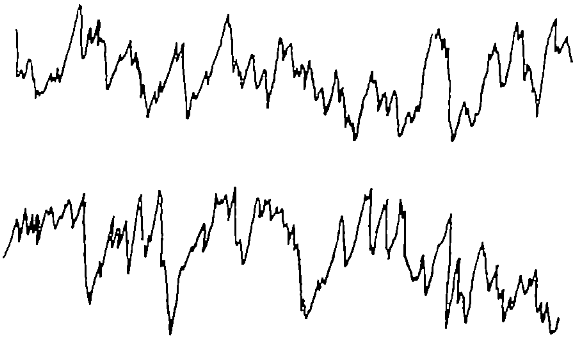

Fig. 2. Time trace of random fluctuation. Recording speed, 10 cm./sec.

A sample of the reactor-power random fluctuation is given in Fig. 2. The recording speed of 10 cm. per sec. was found to be most suitable for the measurement of the effective prompt neutron lifetime. The trace was generated by an optical galvanometer reflecting the beam of a mercury arc lamp on to an ultra-violet sensitive paper. This forms an immediately visible semi-permanent record which may be permanently fixed if required. The galvanometer sensitivity was 20 μamp. per mm. of trace reflexion. The investigation is being continued with a fifteen-speed multi-channel recorder and a range of galvanometers of sensitivity as high as 0·03 μamp. and flat frequency responses of 1–500 c.p.s.

The analysis of the time-trace was carried out on the frequency analyser described by Barber *et al.*[2]. Fig. 3 shows a sample of the amplitude spectra obtained. The amplitude spectrum contains sufficient information to plot the reactor transfer function or the power spectral density of the reactor 'noise' (Fig. 4). A typical set of results is given in Table 1.

Table 1

Frequency (c.p.s.)	Noise current spectral density (relative values)	Noise power spectral density (relative values)
1·06	97·2	2,332
2·00	102·7	2,852
2·94	99·3	2,223
3·88	98·3	2,294
4·82	66·0	1,055
5·76	76·2	1,904
6·70	64·8	992
7·63	67·7	1,158
8·57	56·1	709
9·51	58·0	753
10·45	38·9	335
11·39	51·9	670
12·33	41·3	368

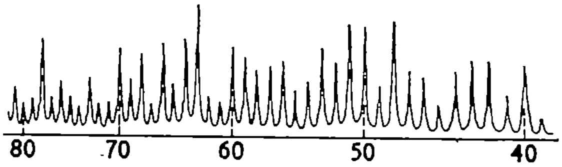

Fig. 3. Amplitude spectrum of random fluctuation, showing peaks from 40th to 80th harmonic of spectrum analyser

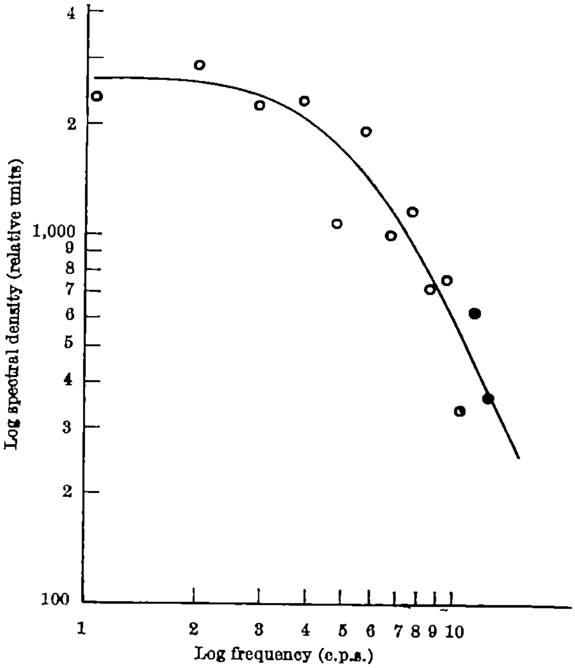

Fig. 4. Random fluctuation spectral density

The effective prompt neutron lifetime in the reactor core was found to be 174 μsec. with an experimental accuracy of 7 per cent. This is consistent with the accuracy of the delayed neutron data available at present. An improvement in accuracy could be obtained by refinements in the electronic circuit and an improvement in the statistics of the analysis through the use of greater lengths of time-trace records. The variation of the neutron lifetime in different parts of the core can be clearly observed with this technique.

A full report will be published elsewhere.

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[1] Moore, M. N., *Nucl. Sci. and Eng.*, 3, 387 (1958).

[2] Barber, N. F., *et al.*, *Nature*, 158, 829 (1946).

Time-dependent Tensile Strength of Solids

Hsaio[1], considering strain-rate effects on polymeric and metallic solids, states that for any given value of strain, the stress obtained at different rates of straining is linearly related to the logarithm of the strain-rate. This is considered to be a basic law which is reasonably safe to apply to a large number of solids. He also presents data for polystyrene showing a linear dependence of fracture stress on the logarithm of strain-rate. These facts are used as evidence supporting certain theories concerning the time-dependence of tensile properties.

The results quoted by Hsaio are confined to a range of two decades of strain-rate in the region of 10^{-5} sec.$^{-1}$. In hitherto unpublished work we have measured the tensile properties of isotactic polypropylene (in the form of thin filaments oriented to a draw ratio of about 5·5 : 1) over six decades of strain-rate from 10^{-4} to 10^{3} sec.$^{-1}$ at 20° C. The dependence of stress at a given strain, and of yield and breaking stress, on the logarithm of strain-rate is shown in Fig. 1. These figures reveal that though the conclusions of Hsaio might be applicable to this material in the range of strain-rates in which he worked, they are certainly inapplicable in a range which extends to higher rates of deformation.

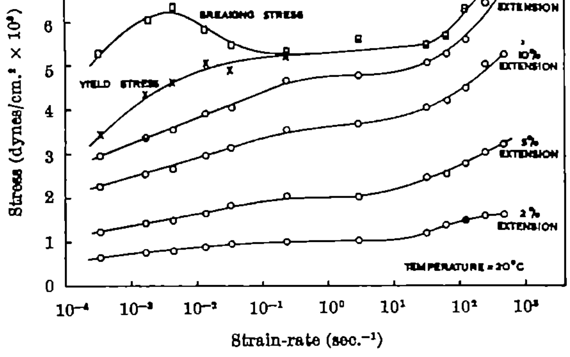

Fig. 1. Effect of strain-rate on tensile properties of oriented isotactic polypropylene

It is intended to publish the full details of this work elsewhere.

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Composition Requirements for Glass Formation in Metallic and Ionic Systems

We have developed in recent papers the point of view that all liquids, ionic and metallic included, would form glasses when sufficiently undercooled, save for the intervention of crystallization[1-3]. We have shown[3] that the glass-forming tendency (for a given rate of cooling and for substances of a given molecular type) is greater the lower is the reduced melting temperature :

$$\tau_m = \frac{kT_m}{h_v} \qquad (1)$$

where T_m is the thermodynamic crystallization temperature, and h_v is the molecular heat of vaporization. This criterion is in semi-quantitative accord with experience on glass formation. In particular it encompasses Zachariasen's[4] criterion, and accounts for the enhancement of glass-forming tendency by molecular asymmetrization, both being simply equivalent to the depression of τ_m. It is also clear, as we pointed out, that the addition of impurities which depress τ_m similarly enhances the glass-forming tendency. In this communication we point out striking new experimental confirmation of this last deduction.

Klement, Willens and Duwez[5] have recently reported the formation of glass in a gold–silicon alloy containing 25 atomic per cent silicon. This appears to have been the first instance in which a metallic glass was formed by cooling the melt. Glass formation in this experiment was, of course, facilitated by the drastic cooling-rate achieved. However, that the gold–silicon alloy in particular formed a glass is, we believe, a consequence of its low τ_m. The composition of the alloy is near the eutectic (reported[6] to be 31 atom per cent silicon) for which $\tau_m \sim 0·015$. Thus its τ_m and that of neighbouring compositions are low enough for a strong glass-forming tendency to exist. A similar strong tendency toward glass formation should be exhibited by gold–germanium alloys of near eutectic compositions and indeed by any eutectic with a similar pronounced dip in the melting temperature.

We infer from the reported room temperature instability that the glass transition temperature, τ_g (see ref. 3 for definition), of the gold–silicon alloy is near room temperature, that is, $\tau_g \sim 0·007$. This is a much lower glass transition temperature than for other fluid types and is a consequence of the generally higher fluidity of liquid metals.

Another unique feature of the gold–silicon glass is its high stability against crystallization at temperatures much above those at which pure amorphous metal films apparently can crystallize. For example, Hilsch[7] has shown that such films may crystallize at temperatures below 20° K. ($\tau \sim 0·001$). The reason for this stability is that, while in the pure material crystallization requires atom motions of less than one atom diameter, crystallization of a eutectic requires chemical diffusive motion over several atom diameters.

In ionic melts one would expect a similar strong glass-forming tendency in low melting eutectics. This expectation is confirmed by the observations of Dietzel and Poegel[8], who showed that the glass-forming tendency of the calcium nitrate–potassium nitrate system is greatest for solutions of near eutectic composition. J. D. Mackenzie (personal communication) has shown that these solutions are mainly ionic in character.

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Ultra-Violet Absorption Spectrum of Water

On further examination, the absorption peaks of water recently discussed[1] have been found to be inherent in the instrumental technique used. The fall in the values of optical density at the lower wave-lengths is the normal stray-light effect under these conditions, but the 'fine structure' is now thought to be due to the oxygen absorption in the factory-sealed monochromator and inadequate compensation within the instrument when the slit automatically opens and closes on passing through the wave-lengths at the oxygen absorption peaks.

The water spectrum in the far ultra-violet to 185 mμ as shown by Barrett and Mansell[2] has been confirmed in this Laboratory by a grating instrument (Optika *O.F.*4) which has just been installed.

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[2] *Nature*, **187**, 138 (1960).

METALLURGY

Microhardness and Other Properties of Magnesium Compounds with the Calcium Fluoride Structure

Relations have previously been proposed[1,2] between hardness and latent heat of fusion, L_f, for pure metals. This communication shows that a relation also holds for the series of intermetallic compounds Mg_2Si, Mg_2Sn and Mg_2Pb, and by using the results for these compounds, L_f for Mg_2Ge is obtained by interpolation.

The four compounds form an isomorphous series from the covalent semiconductor Mg_2Si to the nearly metallic Mg_2Pb. On electrochemical grounds the bond-strength, and therefore also the mechanical strengths, would be expected to decrease in the same order. Fig. 1 shows that this is indeed the case for microhardness, while degree of brittleness (as indicated by cracking near the impression) also decreases in this order. Alloys near the composition of the respective phases were melted and slowly cooled to form large idiomorphic grains; the microstructures of the four alloys were remarkably similar. Impressions were made on a freshly polished surface with a diamond pyramid indenter under 50 gm. load. Great care was necessary in the preparation of these surfaces as all the compounds tarnish rapidly in moist air, the rate of attack increasing from Mg_2Si to Mg_2Pb.

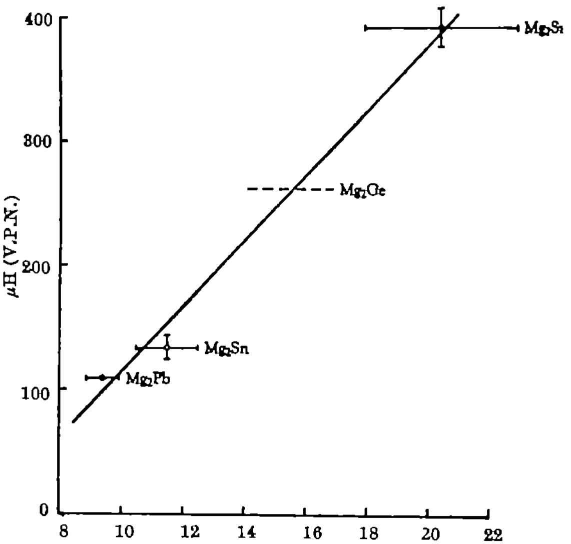

Fig. 1. Relation between microhardness and heat of fusion

In Fig. 1 it may be seen that the hardness-level of Mg_2Ge should intercept the curve at $L_f = 15{\cdot}5$ kcal./mole, and it is suggested that this would be accurate to ± 1·5 kcal./mole. No data have been found in the literature to verify this value.

That these compounds do in fact form a physico-chemical series is shown by Fig. 2, where the microhardness is seen to be a function of the interatomic distance, r, between unlike atoms. The relation is of the form given by Goldschmidt[3]:

$$H = S \cdot \frac{e_A \cdot e_B}{r^m}$$

where S and m are constants, and e_A and e_B the valency of the two atoms. The slope m seems to

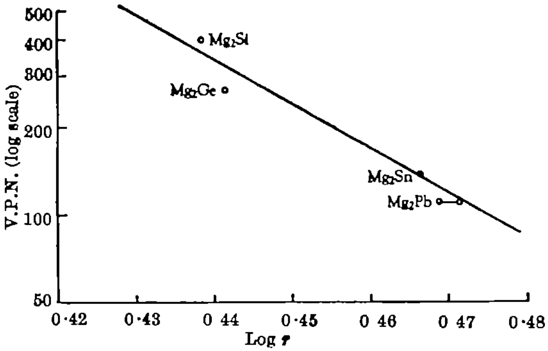

Fig. 2. Relation between microhardness and interatomic distance

Table 1

Group	*m*	Ref.
I–VII (Sodium chloride type)	5	3
IV–IV (Silicon carbide type)	5	4
III–V (Zinc sulphide type)	9	4
III—Transition metals	11	5
II–IV (Calcium fluoride type)	15	Present work

depend on crystal structure and position in the Periodic Table, as is apparent in Table 1. It is hoped that work in hand on a wider range of intermetallic compounds will allow a better understanding of the Goldschmidt rule.

My thanks are due to Mr. R. G. Turner for help with the experimental work, and to Aluminium Laboratories, Ltd., Banbury, for permission to publish this communication.

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Aluminium Laboratories, Ltd.,
Banbury,
Oxfordshire.

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Osipov, K. A., and Fedotov, S. G., *Izv. Akad. Nauk. S.S.S.R. Otd. tekhn. nauk.*, **2**, 98 (1955).

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ENGINEERING

Reactions on Metal Surfaces at High Temperatures

In a recent communication in *Nature*[1] reference was made to a technique developed at Thornton Research Centre for investigating high-temperature reactions between metal surfaces and 'extreme-pressure' lubricating oil additives. We should like to take this opportunity of describing briefly the development of the technique and its potentialities.

Extreme-pressure lubricating oil additives, as their name implies, are used in many applications to protect sliding metal surfaces against incipient seizure (scuffing), under conditions of heavy load when normal hydrodynamic lubrication fails. They are generally considered to function[2] by reacting with the metal surfaces in the contact zone, thus interposing a solid film of low shear-strength between the mating surfaces. The stimulus for reaction comes from the local high temperatures generated in the contact area. Extreme-pressure additives vary in their effectiveness, however, and this is due, at least in part, to differences in their reactivities. Investigating such a hypothesis is complicated by the difficulty of measuring the reactivities of relatively unstable additives at temperatures comparable to those generated transiently in practice.

This difficulty was partially overcome by simulating the high metal temperatures in a static system by electrical heating. The principle was simple and merely involved passing a current through a thin metal wire immersed in an oil containing an extreme-pressure additive. Metal temperatures were deduced from the measured resistance/temperature characteristics of the metal. Reaction-rates at various temperatures were followed from the increase in resistance of the wire due to chemical attack by the additive. The method was successful but had one limitation: the maximum temperature which could be attained was determined to a large extent by the boiling point of the oil. This meant that maximum temperatures in general were limited to about 600° C., even in oils of high boiling point. This limitation was overcome by passing short pulses of current through the wire. The duration of the pulse was selected so that the fluid surrounding the wire did not vaporize to any appreciable extent during this period. In this way, temperatures up to the melting point of the wire could be easily reached in both volatile and non-volatile solvents. The duration of a single pulse, and hence the minimum reaction period, was 10^{-3} sec.

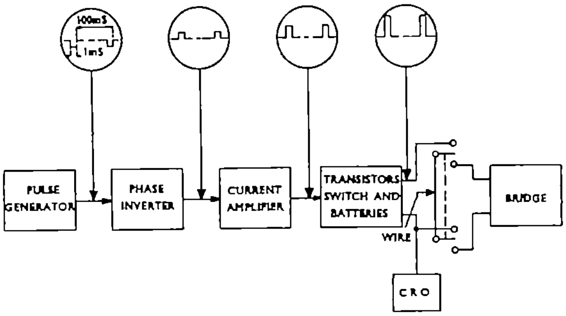

Fig. 1. Block diagram of hot-wire pulsing equipment

The heart of the system is the electronic device for the generation and control of the pulses. How this was achieved is shown diagrammatically in Fig. 1. Since the voltage across the wire is a square wave, the current wave-form is defined by the increase in resistance of the wire due to its rise in temperature. The current wave-form, observed on a suitable oscilloscope, can thus be used to determine the temperature of the wire. Reactions between additive and metal can be followed by estimating the thickness of reaction product by radiotracer methods, X-ray diffraction, etc., or more simply from increases in resistance of the wire, as mentioned previously. We have used the latter method.

Some results obtained with this pulse technique using a stainless steel wire are shown in Fig. 2. The plot of $\log_{10}$ initial reaction-rate against $1/T$(° K.) is linear and shows clearly the effect of temperature on the reaction-rates. Reactions of carbon tetrachloride (boiling point 77° C.) were as easy to study as those of dibenzyl disulphide in medicinal white oil (boiling point approx. 450–500° C.) The products

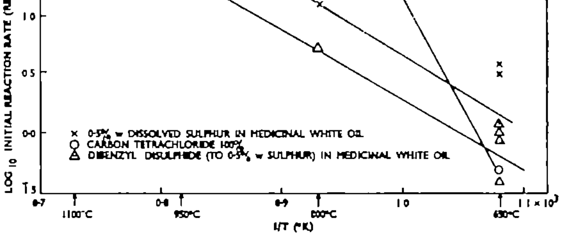

Fig. 2. Reactivity of extreme-pressure additives at different temperatures

of reaction, identified by X-ray diffraction, were: FeS for the sulphur additives and $FeCl_2.4H_2O$ for carbon tetrachloride.

The technique is versatile, rapid and simple to use, and certainly achieves temperatures comparable to those considered to be generated transiently on meshing gear surfaces, for example. These studies of reactions between extreme-pressure additives and metal surfaces at high temperatures are giving us a better understanding of the mechanism of action of extreme-pressure lubricants. A more detailed account will be published elsewhere.

We wish to thank the Directors of "Shell" Research, Ltd., for permission to publish this communication.

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CHEMISTRY

Use of Krypton for Surface Area Measurements

CONSIDERABLE interest has recently been focused on the suitability of using krypton adsorption isotherms at liquid air temperatures for the determination of the surface area of solids. From the isotherms the monolayer volume, v_m, which can be converted to surface area using the effective cross-sectional area of an adsorbed krypton atom, is obtained by the Brunauer–Emmett–Teller method[1]. This method generally yields a linear plot of $p/v(p^0 - p)$ against p/p^0 (v = volume adsorbed at an equilibrium krypton pressure, p; p^0 = s.v.p. of krypton at the adsorption temperature) in the range of relative pressure, p/p^0, 0·05–0·35.

Haul[2] considers that krypton is more suitable than nitrogen as adsorbate for measuring small surface areas. Malden and Marsh[3], however, state that nitrogen should be used in preference to krypton as the latter is liable to yield Brunauer–Emmett–Teller plots which are convex to the p/p^0 axis. These curved plots greatly reduce the accuracy of the determination of v_m. More recently, Gaines and Cannon[4], on the basis of a large number of area determinations and on theoretical grounds, consider that the general applicability of krypton, even for p/p^0 ranges extending well below 0·05, has been amply demonstrated. This conclusion is supported by recent work on evaporated iron films[5,6]. Krypton adsorptions on these films at liquid nitrogen temperature yield Brunauer–Emmett–Teller plots which are accurately linear in the p/p^0 range 10^{-4} to 5×10^{-2} and give meaningful v_m values. In the light of the foregoing remarks, the following results may be of interest.

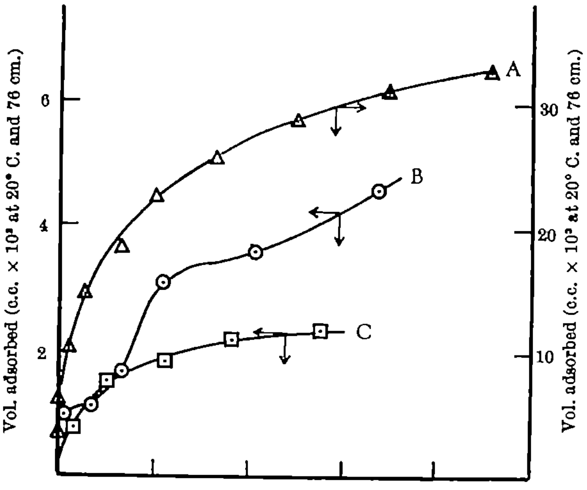

Fig. 1. Krypton adsorption isotherms. *A* (at −193·2° C.), fresh film, $v_m = 33·5 \times 10^{-3}$ c.c. at S.T.P.; *B* (at −192·8° C.), sintered film, $v_m = 5·24 \times 10^{-3}$ c.c. at S.T.P.; *C* (at −193·0° C.), 'treated' film, $v_m = 2·47 \times 10^{-3}$ c.c. at S.T.P.

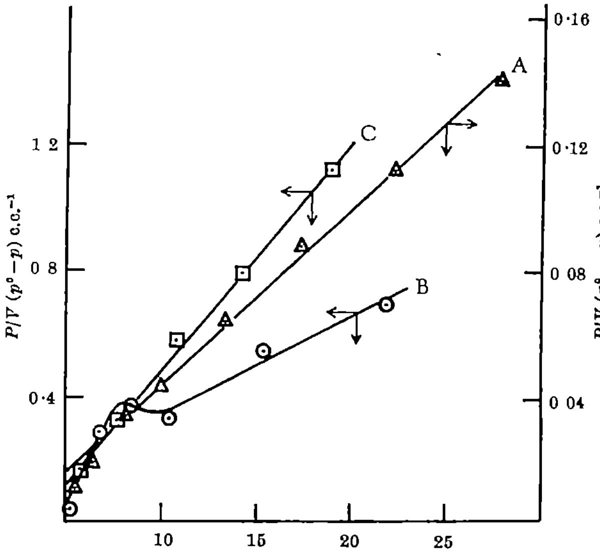

Fig. 2. Brunauer–Emmett–Teller plots from data shown in Fig. 1

A chemically clean silver film was produced in high vacuum ($< 10^{-6}$ mm. mercury) by evaporation on to the walls of a 'Pyrex' vessel the outside temperature of which was kept at − 193·3° C. This film, which weighed 10 mgm., gave the familiar σ-shaped krypton adsorption isotherm at − 193·2° C. and a linear Brunauer–Emmett–Teller plot (curves *A*, Figs. 1 and 2). Sintering the film at 20° C. for 90 min. caused the shape of the next low-temperature isotherm at − 192·8° C. to alter radically (curve *B*, Fig. 1). Moreover, the Brunauer–Emmett–Teller plot is anomalous, being composed of two approximately linear portions separated by a kink (curve *B*, Fig. 2). Curves *C* (Figs. 1 and 2), both of which are normal, were obtained at − 193·0° C. after the sintered film had chemisorbed oxygen at temperatures up to 20° C. followed by prolonged pumping (17 hr. at $< 10^{-6}$ mm. mercury) at room temperature.

For the freshly prepared and 'treated' silver surfaces, monolayer volumes deduced from the Brunauer–Emmett–Teller plots in the p/p^0 range 5×10^{-4} to 5×10^{-3} were in good agreement with those estimated roughly from the isotherms (*A* and *C*, Fig. 1). For the sintered silver surface, uncertainty exists as to which of the two straight lines in the Brunauer–Emmett–Teller plot should be taken as the basis for obtaining v_m. It does, however, emerge that the

v_m obtained from the slope of the Brunauer–Emmett–Teller straight line at the higher p/p^0 range 1×10^{-3} to $3{\cdot}4 \times 10^{-3}$ (curve *B*, Fig. 2) agrees to within 25 per cent with the v_m estimated roughly from curve *B*, Fig. 1, on the assumption that adsorption corresponds to monolayer completion at the last recorded pressure.

Other silver films prepared and sintered in the same way as the one described above gave essentially the same results as those shown in Figs. 1 and 2. In all instances, Brunauer–Emmett–Teller plots are anomalous for a freshly sintered surface over the p/p^0 range 5×10^{-4} to 5×10^{-3}, at which normal linear Brunauer–Emmett–Teller plots are obtained for a freshly evaporated or 'treated' silver surface.

Anomalous Brunauer–Emmett–Teller plots, similar in shape to those described here, have been reported previously on polycrystalline graphite[7] and, to a smaller degree, on evaporated carbon films[8]. No adequate explanation for such anomalies has yet been found, though it is significant that curve *B*, Fig. 1, suggests that vertical steps in the isotherm, and hence second-order phase changes in the adsorbed film, may be present.

From a practical point of view, however, the occurrence of anomalous krypton Brunauer–Emmett–Teller plots on surfaces which have not chemisorbed any gases but have simply been sintered reduces the power of the krypton adsorption method of measuring small surface areas. A further account of this work will be published elsewhere.

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Reaction of Oxygen Atoms with Hydrogen

THE reaction between ground-state oxygen atoms and hydrogen:

$$O(^3P) + H_2 = OH + H\,;\ \Delta H^0 = 0\ \text{kcal./mole} \qquad (1)$$

is known to be an elementary process in the branching chain reaction between hydrogen and oxygen, and it is therefore of interest to determine its kinetics. Harteck and Kopsch[1] measured the amounts of water formed in the reaction between hydrogen and electrically discharged oxygen containing about 25 per cent of oxygen atoms at three temperatures between 300° K. and 462° K. From these data an activation energy of 6 ± 1 kcal./mole for reaction (1) was computed. However, the formation of water under these conditions may arise not only from reaction (1) followed by reaction (2):

$$OH + H_2 = H_2O + H\,;\ \Delta H^0 = -13\ \text{kcal./mole} \qquad (2)$$

but also from a chain reaction involving molecular oxygen. In the absence of undissociated oxygen, the only secondary reactions to be considered are reactions (2) and (3):

$$O + OH = O_2 + H\,;\ \Delta H^0 = -14\ \text{kcal./mole} \qquad (3)$$

The velocity constant of reaction (2) has been determined as $1{\cdot}2 \times 10^{14} \exp(-10{,}000/RT)$ cm.^{3} mole^{-1} sec.$^{-1}$ by Avramenko and Lorentso[2], and a value of $k_2 = 2 \times 10^{12} \exp(-2{,}000/RT)$ cm.^{3} mole^{-1} sec.$^{-1}$ can be obtained from Semenov's value[3] for the rate constant of the reverse reaction and from the calculated equilibrium constant.

There is evidence that ^1D oxygen atoms react more rapidly with hydrogen than do ^3P ground-state atoms[4–6], and it is also possible that the value of k_1 determined by Harteck and Kopsch[1] was affected by the presence of excited atoms in the discharged oxyen.

Oxygen atoms in the absence of molecular oxygen can be produced by addition of nitric oxide to nitrogen atoms in active nitrogen, when the stoichiometric and very rapid reaction (4) occurs[7–9]:

$$N(^4S) + NO(^2\Pi) = N_2(^1\Sigma) + O(^3P)\,;$$
$$\Delta H^0 = -75\ \text{kcal./mole} \qquad (4)$$

Tanaka *et al.*[10] have shown that the concentration of excited nitrogen atoms in active nitrogen is about 1/500 of that of ground-state atoms, and the high collision efficiency of reaction (4) (ref. 11) shows that it does not violate the spin correlation rules. It therefore follows from a consideration of the energies of the various electronic states of N_2 and O that both these species are formed in their electronic ground-states.

In this work active nitrogen was produced by passing purified nitrogen through an 18 Mc./sec. electrodeless discharge. Nitric oxide was added past the discharge in slight excess (about 5 per cent excess), as shown by the appearance of a weak air afterglow continuum ($NO + O = NO_2 + h\nu$) downstream from the mixing point. The nitrogen, now containing about 0·5 per cent of oxygen atoms, passed through a 21-mm. diameter quartz tube enclosed in a furnace. This tube had four mixing inlets spaced at 30 cm. intervals, through which hydrogen could be introduced[12]. Oxygen atom concentrations in the gas leaving the furnace were determined by measurement of the intensity of the air afterglow produced when known flow-rates of nitric oxide were added 10 cm. upstream from a photomultiplier tube, 30 cm. downstream from the furnace exit. The intensity of the air afterglow is known to be proportional to both oxygen atom and nitric oxide concentrations[13], and this process is unaffected by the presence of hydrogen atoms (unpublished results).

In this system the rate of removal of oxygen atoms by the third-body recombination reactions $O + O + M = O_2 + M$ and $NO + O + M = NO_2 + M$ is much smaller than the rate of reaction of oxygen atoms with hydrogen, and the correction to the data to allow for these reactions was less than 5 per cent. The rate of reaction of oxygen atoms was found to be first order in both oxygen atom and hydrogen molecule concentrations between 409° K. and 733° K. and at total pressures from about 1·5 to 3 mm. mercury. There was no evidence that the reaction-rate depends on total pressure, and the only third-order reaction which could be important in our system:

$$O(^3P) + H_2 + M = H_2O + M$$

has an apparently negligible rate, presumably because it violates the spin correlation rules. In our experiments $k_3(O)$ is always greater than $k_1(H_2)$ or $k_1(H_2)$ and the observed rate constant is therefore $2k_1$. Our results at six temperatures between 409° K. and 733° K. are represented by $k_1 = 1{\cdot}2 \times 10^{13} \exp(-9{,}200/RT)$ cm.^{3} mole^{-1} sec.$^{-1}$ to within 10 per cent. Measurements of the rate of reaction of oxygen atoms with deuterium are being made by the same technique.

An attempt is being made to investigate the kinetics of reactions (2) and (3) and thus to confirm the results of Avramenko and Lorentso[2], and of Semenov[3].

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Origin of Some Primary Coal Tar Components

THE presence in the ether-, chloroform- or light petroleum-soluble portions of extracts from bituminous coals of paraffinic, alicyclic and alkylated aromatic hydrocarbons was demonstrated between 1910 and 1920[1-5]. Pictet and Bouvier first showed the presence of similar compounds in a vacuum tar[6], while Jones and Wheeler found the pyridine-soluble, chloroform-soluble extract of a bituminous coal to be the main source of hydrocarbons and resins, the former being characterized as paraffins, olefins and naphthalenes[4].

On the basis of a review of the literature, Dryden and others[7] contended, unlike me[8], that no tar was really primary in the sense that it contained materials related structurally to the coal, but that small, reactive fragments were first formed by pyrolysis, which built up to the more complex constituents of a tar. A 'primary' tar was one only in the sense that it contained substances formed by a relatively simple sequence of reactions. Subsequent experimental work carried out by this school, from which resemblances were found between chloroform extracts of coals heated to the plastic range and the volatile products of their vacuum pyrolysis, appears to have modified their original view. The existence of relatively primary pyrolysis products is now admitted[9], but the techniques they used did not result in the separation of hydrocarbons, and the hypothesis was put forward that these pyrolysis products were formed entirely by depolymerization of the coal.

A recent mass spectrometric study of coal vitrains by Holden and Robb confirms the earlier views that hydrocarbons contained in the coal are distilled off on heating, for the following were evolved before the main decomposition had set in: aliphatics of molecular weight 200–350, containing much cyclic material; and alkyl aromatics, including alkyl-substituted benzenes, naphthalenes and higher members. These compounds were thought to be bound in the coal in a loose, physical way, rather than chemically, and to be similar to those from chloroform-soluble extracts of coals[10].

It must, therefore, be taken as established that primary tars contain similar compounds to coal extracts containing lower molecular weight material. It has not been shown, however, how close is this relationship, qualitatively and quantitatively.

I have shown that four broad fractions were invariably present in the neutral, light petroleum-soluble portions of primary tars made from coals of low rank[11]. Their separation was later standardized as part of a method of analysis of tars based on solvent extraction and chromatography[12]. Preliminary qualitative work is here reported, undertaken to establish whether similar fractions were present in a bituminous coal as in the primary tar derived from it.

The tar was made by internal heating with hot gas of the coal[13] (Thoresby coal: orthohydrous, weakly caking, high-volatile bituminous). The neutral, benzene-soluble, light petroleum (40–60°)-soluble portion was subdivided by adsorption chromatography on silica gel with eluants in the following

Table 1. COMPARISON OF FRACTIONS FROM COAL EXTRACT AND COAL TAR

Fraction		Description applying to each pair of fractions	% yield on dry extract or tar	% yield on dry, ash-free coal	Predominant chemical types found in coal tar fractions, *b*	% C	% H	%(O+)	C/H	Molecular weight	n_D^{20}
1	*a*	White, soft wax, consisting of mixture of oils and waxes	4·13	0 14	*n*-, *cyclo*- and *iso*-paraffins; highly alkylated benzenes	86·0	13 9	0·1	0·51	240	1·474 (25°)
	b		6 75	0 61		85 3	13·6	1 1	0·52	240	1·458 (25°)
2	*a*	Yellow, mobile oil	3 09	0·11	Alkylated hydronaphthalenes	87·7	11 8	0 5	0 62	240	1 584
	b		13 48	1·21		87·2	11·0	1·8	0 66	220	1·525
3	*a*	Dark brown, viscous oil	17 32	0·61	Alkylated polycyclics, mainly naphthalenes	90 6	8 9	0·5	0·85	220	1·613
	b		20 57	1 85		87·5	8 3	4 2	0 88	230	1·610
4	*a*	Dark brown, semi-solid, with characteristic odour	4·74	0·17	Complex mixture of oxygenated aromatic compounds	77 2	9·0	13·8	0 72	360	—
	b		5 02	0 45		77 2	8 4	14 4	0 77	325	—

a, Coal extract fraction; *b*, coal tar fraction; (O+), oxygen and other elements by difference.

order: light petroleum (40–60°) (two fractions, the first non-fluorescent, the second fluorescent in ultra-violet light); benzene (one fraction); and ethanol (one fraction)[13]. Work is in progress on the characterization of these fractions, part of which has been published[14].

The same coal, sized between ¼ and ½ in. screens, was extracted with a benzene–ethanol mixture (70–30) for more than 50 hr. in a large Soxhlet apparatus until the percolate was virtually colourless. The yield of extract was 3·19 per cent on the coal, air-dried basis, equivalent to 3·50 per cent on the dry, ash-free basis. It is realized that these extractions were incomplete, but the method used enabled material to be obtained in sufficient quantity for a qualitative evaluation. The neutral, light petroleum-soluble portion of this extract, obtained by exhaustive extraction, was similarly subdivided by chromatography.

Table 1 gives data for the chromatographic fractions from both the coal and the coal tar. The similarity between the corresponding pairs of fractions in chromatographic behaviour, appearance, analysis and physical properties is striking. The yields of the coal extract components are less in each case than the corresponding ones from the coal tar.

The oxygen contents of all the coal tar fractions are higher than those from the coal, presumably due to the greater oxidation occurring during preparation and storage of the tar. The infra-red absorption spectra of the two fractions 4 have been found to be almost identical, both indicating the presence of free and chelated OH, aromatic structures and much alkylation. These two fractions oxidize and polymerize readily; thus, the molecular weight of that from the coal increased from 360 to 520 after storage for a few months.

This preliminary work has shown that an important part of the simpler, neutral constituents of a primary tar exists as such in the original coal. It still remains to be shown whether completer extraction of the coal will account for the whole of the remainder of the neutral, light petroleum-soluble constituents of the tar, or whether part of the latter is formed by decomposition of the coal.

I wish to thank Mrs. A. Sen Gupta and G. V. Blunt for help with the experimental work.

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Organic Halides as Electron Acceptors in Charge Transfer Compounds

IN the literature several indications of the existence of directed chemical bonds between organic halogen compounds and molecules containing atoms like nitrogen, oxygen or sulphur may be found. Results arrived at during the past few years concerning the structure of addition compounds formed by halogen molecules and molecules of the latter type[1] made it appear desirable to determine the structures also of molecular compounds formed by halides.

X-ray crystallographic structure determinations have been undertaken in this laboratory of a series of solid addition compounds between electron acceptors, like iodoform or oxalyl chloride (bromide), and electron-donating molecules like dioxan, dithiane, amines and sulphur. It soon became clear that iodoform preferably makes use of the iodine atoms for the bonding to the atoms of the partner molecule exhibiting electron-donor properties. Furthermore, it appeared that the atomic arrangement—donor atom–halogen–carbon—is at least approximately linear. In some instances the bond formation results in chains extending through the entire crystal of alternating donor and acceptor molecules (for example, in dithiane-iodoform and dioxan-oxalyl halide).

Often the charge-transfer bonds are evidently very weak. Until now we had no proof of carbon–halogen distances differing from the corresponding ones in the free acceptor molecule, similar to the increase in the halogen–halogen distance observed in many addition compounds where a halogen molecule acts as the acceptor[1].

The bond distance halogen–donor atom is shorter in general than the corresponding sum of the accepted van der Waals radii, but the shortening is in some cases rather small (O—Cl, O—Br).

Representative donor–acceptor distances observed are listed in Table 1 and compared with the corresponding van der Waals values[2]. The figures given may be subject to some, but not significant, corrections after further refinements of the respective structures.

Table 1

Compound	Donor–acceptor	Donor–acceptor distance (Å.)	Sum of van der Waals radii (Å.) (ref. 2)
Quinoline-iodoform (3 . 1)[7]	N—I	2·99	3 65
S_8-iodoform (3 . 1)[8]	S—I	3·44	4 00
Dithiane-iodoform (1 : 1)[8]	S—I	3 29	4 00
Dioxan-oxalyl chloride (1 : 1)[9]	O——Cl	3 18	3 20
Dioxan-oxalyl bromide (1 : 1)[9]	O——Br	3 21	3·35

Molecules containing both donor and acceptor atoms would be expected to crystallize by forming charge-transfer bonds between the molecules. This is certainly the case in solid cyanogen bromide and iodide. Crystals of these compounds have been shown[3,4] to contain linear arrangements —Hal–C–N—Hal–C–N—. The structure determinations do not allow exact bond distances to be given. The chains, however, are directed along crystallographic axes and some conclusions may therefore be drawn from the observed lattice constants. If it may be assumed that the Hal–C and C–N distances do not differ significantly from the distances found in the free molecules (microwave measurements[5,6]), it follows that the Hal—N distances are equal to, or shorter than, 2·85 Å. (Br) and 2·80 Å. (I), with an uncertainty probably not exceeding 0·1 Å. Neutron diffraction studies of the cyanogen halides are being planned.

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BIOCHEMISTRY

Enzymic Synthesis of 3-O-α-D-Glucopyranosyl D-Xylose

Transglycosylation reactions with microbial enzymes offer a facile method for the synthesis of new oligosaccharides. Following our earlier report[1] on the production of 3-*O*-β-D-glucopyranosyl D-xylose by the action of a cell-free extract of *A. niger* 152 on a cellobiose–xylose mixture, the corresponding disaccharide containing an α-glucosidic linkage has now been synthesized via the following reaction:

maltose + xylose → 3-*O*-α-glucosyl-xylose + glucose

catalysed by a transglycosylase from *Penicillium lilacinum*.

Mycelia of *Penicillium lilacinum* grown on mineral media (magnesium sulphate heptahydrate 0·074 gm., ammonium nitrate 0·1 gm., potassium dihydrogen phosphate 0·139 gm. and sodium chloride 0·001 gm. in water, 300 ml.) containing maltose (50 gm.) were harvested, washed, shredded in an Atomix and freeze-dried. A cell-free enzyme extract (3·5 gm.) prepared by disintegration of an aqueous suspension of the cells in a Mickle tissue disintegrator was incubated at 30° for 5 days with a sterile aqueous solution (150 ml.) of maltose (30 gm.) and xylose (15 gm.). After arresting enzyme action, the mixture of sugars formed was separated by chromatography on sheets of Whatman No. 3 irrigated with the organic phase of a butanol/ethanol/water (4 : 1 : 5) mixture. The band R_{maltose} 1·75 suspected to contain a reducing glucosyl–xylose was eluted and freeze-dried. Acidic hydrolysis (2 *N* sulphuric acid at 100° for 2 hr.) and paper chromatographic analysis of the hydrolysate confirmed the presence of glucose and xylose in the new component and its disaccharide character was established from the mobility[2] (*M* 0·8) of its N-benzylglycosylammonium ion. Reduction of the disaccharide (2 mgm.) with sodium borohydride (2 mgm.) and acid hydrolysis of the resulting disaccharide alcohol yielded a mixture of glucose and xylitol. Hence the disaccharide must have been a glucosyl–xylose. Its optical rotation ($[\alpha]_D^{18}$ + 87·5°, conc., 0·70 in water) and the presence of an absorption peak at 850 cm.$^{-1}$ in its infra-red spectrum established[3] that the glucosidic linkage was of the α-type. The instability to alkali of the disaccharide and the ionophoretic mobility of its borate complex[4] (M_G 0·68–0·70) both suggested that the junction of the two monosaccharide units was 1 → 3. Furthermore, the positive reaction of the disaccharide with the triphenyltetrazolium chloride reagent[5] ruled out a linkage of the 1 → 2 type. The disaccharide (9 mgm.) was treated with 4 per cent methanolic hydrogen chloride (0 5 ml.) at room temperature for 1½ hr. and the resulting methyl furanosides (6·5 mgm.: $[\alpha]_D^{18}$ + 74°, conc., 0·3 in water) isolated. Treatment with sodium metaperiodate (10 mgm. in 2 ml water) at room temperature for 36 hr., followed by addition of ethylene glycol (0·1 ml.), freeze-drying and passage down a column (15 × 1 cm.) of 'Ultrasorb', gave the salt-free periodate oxidized product (3 mgm.). Reduction of this with sodium borohydride (4 mgm.) yielded a polyalcohol containing an intact xylosyl moiety. This was demonstrated by acidic hydrolysis (*N* hydrochloric acid at 100° for 1 hr.) to yield xylose, characterized by the identity of its infra-red spectrum with that of an equilibrium mixture of an authentic specimen of D-xylose. This last experiment confirmed that the xylose moiety was linked through the 3 position and that the enzymatically synthesized disaccharide was 3-*O*-α-D-glucopyranosyl D-xylose.

One of us (D. B. E. S.) thanks the University of Birmingham for the award of a scholarship.

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Applicability of Hydroxocobalamin as a Long-acting Vitamin B12

The main difficulty in the treatment of vitamin B_{12} deficiencies has been that small doses of cyanocobalamin are inadequate to replenish the body's depleted stores and that most of the large parenteral doses are eliminated in the urine within the first 12 hr.

Consequently, attempts have been made to develop a long-acting vitamin B_{12} which could be retained in the body more efficiently than cyanocobalamin[1] by combining it with sesame oil and aluminium monostearate or by making it into a zinc–tannate complex[2].

In the search for a long-acting B_{12} preparation, we screened cobalamins in various media and in various modifications. In these investigations, which will be reported in detail elsewhere[3], only an aqueous solution of hydroxocobalamin (kindly supplied by Dr. Nathaniel S. Ritter, of the Merck Sharp and Dohme Research Laboratories, Division of Merck and Co., Inc., Rahway, N.J.) was found to possess all the features sought for in a long-acting vitamin B_{12}.

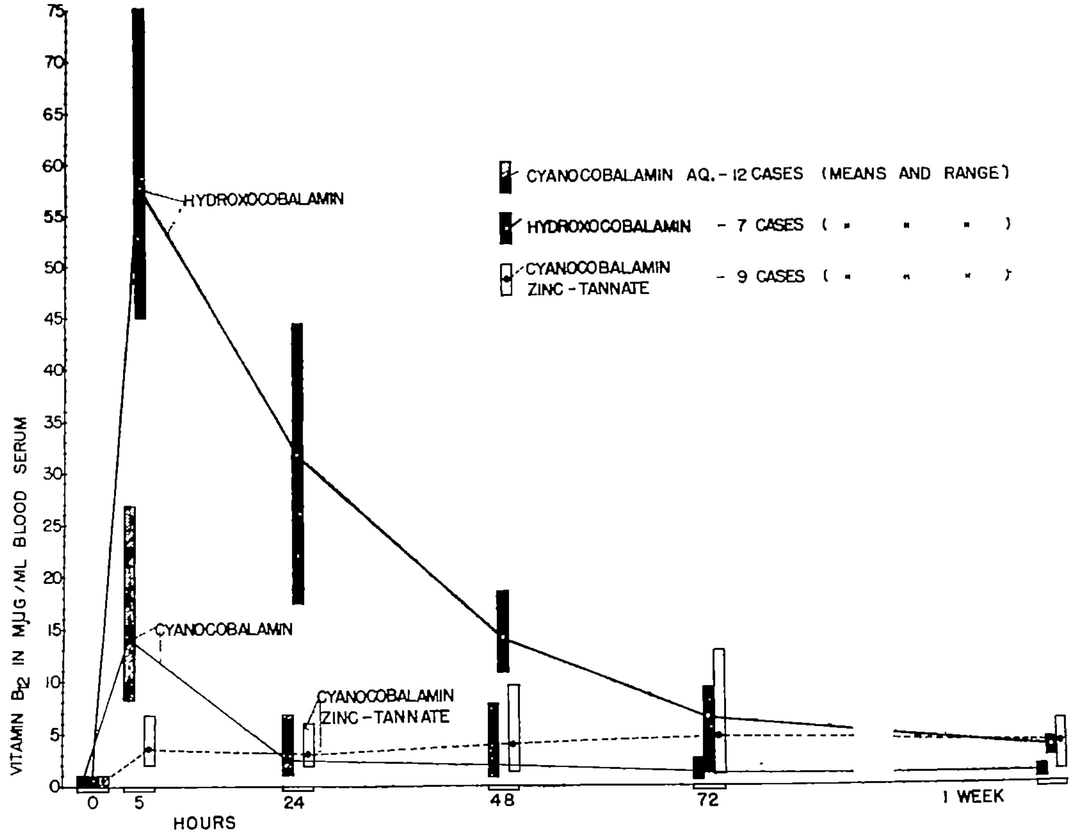

Fig. 1. Vitamin B_{12}-levels in blood serum following intramuscular injection of 1,000 μgm. cyanocobalamin, hydroxocobalamin and cyanocobalamin zinc – tannate

Since hydroxocobalamin (formerly known as vitamin B_{12a} and B_{12b}) exerts a hæmatopoietic activity approximately equivalent to that of cyanocobalamin in man[4], we made a detailed study of its absorption, deposition and excretion, and summarize here some of the results obtained.

Seventeen patients with normal B_{12}-levels in the blood and no evidence of circulatory or renal failure received hydroxocobalamin in aqueous solution; nineteen, cyanocobalamin in aqueous solution; and seventeen, cyanocobalamin zinc–tannate complex. These materials were injected in a single dose of 500 or 1,000 μgm. Blood was withdrawn, just prior to injection, and at frequent intervals afterwards.

Specimens of urine were collected during the three days after injection. In addition, 24-hr. urine specimens were obtained on the seventh, fourteenth, twenty-first and twenty-eighth days following injection. Determination of the total vitamin B_{12} values in blood serum and urine was made by *Lactobacillus leichmannii* assay[5]. In addition, six patients received alternate intramuscular injections of radioactive cyanocobalamin and hydroxocobalamin labelled with cobalt-57 and -58. For two weeks after injection, the sites of injection and several cutaneous projections of the liver were scanned for radioactivity[6] by means of a scintillation detector and gamma spectrometer.

It was found that a single intramuscular injection of 1,000 μgm. hydroxocobalamin results in an average 96-fold rise in the vitamin B_{12}-level in blood serum by the fifth hour, a 52-fold rise by the twenty-fourth hour, a 23-fold rise by the forty-eighth hour and a 10-fold rise by the seventy-second hour. For a 3-week period, the levels of B_{12} in the blood serum were significantly higher, statistically, after intramuscular injection of a single dose of 1,000 μgm. of hydroxocobalamin than after an identical dose of cyanocobalamin. While the initial levels in the blood did not show any statistically significant difference, the mean levels were 1·8 and 4·1 times higher, respectively, 5 hr. after injection of 500 or 1,000 μgm. of hydroxocobalamin, 4·6 and 12·8 times higher by the twenty-fourth hour, 2·4 and 5·2 times higher by the seventy-second hour, and 1·6 and 2·5 times higher by the second to fourth week, than after administration of identical doses of cyanocobalamin (Fig. 1). These differences were highly significant statistically, the *P* value in all instances being less than 0·001.

Compared with identical doses of cyanocobalamin zinc–tannate complex, the mean levels in blood following intramuscular administration of 1,000 μgm. of hydroxocobalamin were 15·5 times higher by the fifth hour, 10·8 times higher by the twenty-fourth hour and 3·6 times higher by the forty-eighth hour (Fig. 1). Statistically, these differences were highly significant.

Study of the urinary excretion of vitamin B_{12} following administration of cyanocobalamin and hydroxocobalamin showed that aqueous hydroxocobalamin was excreted in the urine much less rapidly than aqueous cyanocobalamin. After a single intramuscular injection of 500 or 1,000 μgm. hydroxocobalamin, only 16 and 27 per cent, respectively, are lost in the 72-hr. urines, while the corresponding losses of cyanocobalamin at equal doses and time-intervals amount to 60 and 69 per cent, respectively, of the amount injected.

Absorption of radioactive hydroxocobalamin from the site of injection is much slower than that of an aqueous solution of radioactive cyanocobalamin. Comparative studies of six individuals injected on opposite sides of the body with both materials indicate that, by 24 hr., only 0·7–2·0 per cent (and after 6 days, only 0·5–1·0 per cent) of the cyanocobalamin injected remains at the site of injection, whereas after the same time-interval, 6·0–12·0 per cent and 2·5–4·0 per cent, respectively, of the hydroxocobalamin can still be traced at the injection site. At the end of the 7–14-day interval, the hepatic deposition of radioactive hydroxocobalamin was the same as, or slightly higher than, that following injection of an equivalent dose of radioactive cyanocobalamin in the same patient.

Why hydroxocobalamin is absorbed slowly from the site of injection, why it is retained for a longer period in the body and builds up consistently high B_{12}-levels in the blood, may be explained by its greater reactivity and the formation of stronger bonds with substances in the body which bind B_{12}. This might have been suggested by the results of previous investigations into the binding of hydroxocobalamin to serum proteins[7].

The activity of hydroxocobalamin in its native state, and the higher levels at which it is maintained in the blood, make it a more desirable source of vitamin B_{12} for human use than artificial cyanocobalamin complex depot preparations. Moreover, since the equivalence of the hæmatopoietic effect of hydroxocobalamin to that of cyanocobalamin has been established[4], and its more effective retention by the body, at consistently higher levels in the blood, has been demonstrated, cogent arguments exist in support of the claim of the superiority of hydroxocobalamin over cyanocobalamin for the treatment of vitamin B_{12} deficiencies.

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Later Stages of Protein Synthesis and the Role of Phospholipids in the Process

Recent work in our laboratory[1,2] has shown that lipo-amino-acid complexes are rapidly formed from amino-acids by protoplasts of *B. megaterium*, and further that these complexes have many of the properties to be expected of intermediates in protein biosynthesis. Related studies have also been reported by other workers[3–5]. We have also found that the membrane complex derived by lysis of the same protoplasts under defined conditions contains the complete apparatus of protein biosynthesis[6]. A more detailed examination[7] of this membrane complex has now shown that the bulk of the labelled protein formed after very short periods of incubation of the complex with labelled amino-acids (even a few seconds) is distributed between a phospholipoprotein fraction and ribosomal particles. However, the greater part of the labelled protein is found on the phospholipoprotein fraction, and detailed kinetic experiments[7] carried out with both whole protoplasts and the membrane complex indicate that this is both the main site of initial protein formation and that the protein formed in this fraction is passed on to form soluble protein. In this connexion, it is noteworthy that Dr. P. N. Campbell (personal communication) has evidence indicating that serum albumin synthesized in microsomes of rat-liver is formed on the membrane rather than the ribosomal component of the microsome, and it has been reported[8] that a highly labelled phospholipoprotein is rapidly formed from leucine labelled with carbon-14 by 'ribosomes' from pea-seedling cells.

On the other hand, the evidence that ribonucleic acid (RNA) plays an active part in protein biosynthesis is overwhelming, and it is the purpose of this communication to provide a hypothesis whereby these surprising results may be reconciled with current ideas on the mechanism of protein biosynthesis as outlined by Zamecnik[9] and others. After the initial stage of amino-acid activation, it seems probable from recent work[10] that there exists one RNA of low molecular weight (S-RNA) that acts as a specific receptor for each activated amino-acid, and it further seems likely[11,12] that the S-RNA–amino-acid complexes formed are transferred to an RNA molecule of high molecular weight. This RNA of higher molecular weight can be obtained in the ribosomal particles after suitable cellular fractionation has been carried out, but the work of Hanzon, Hermodsson and Toschi[13] indicates that this RNA may exist in the cell in a more extended form, a conclusion consistent with our own findings in *B. megaterium*[7]. However, for simplicity, it will be referred to here as ribosomal RNA.

The triplet hypothesis of Crick, Griffith and Orgel[14] provides a mechanism whereby the ribosomal RNA can bind and distinguish between the different S-RNA–amino-acid complexes; but a difficulty has been that the S-RNA molecules appear to be too long and complex to serve in their entirety as suitable transfer agents. However, it must be pointed out that this difficulty arises solely because it has been thought that the protein is first formed along the surface of the ribosomal RNA. If, instead, the protein is first synthesized along a phospholipid surface, this difficulty does not arise. We should like to suggest the following possibilities:

(1) That all the S-RNA chains have the same length from the point of triplet attachment to the amino-acid moiety at the end of the chain.

(2) That a phospholipid chain (presumably a phospholipoprotein membrane in most cases) is sited at a distance equal to this length from the ribosomal RNA.

(3) That the amino-groups of the amino-acid moieties can thus each make contact with only one phosphate group of the phospholipid chain. Therefore, although the phospholipid molecules themselves need possess no specificity, the sequence determined by the specific triplet attachment of the two types of RNA molecule is reproduced exactly on the phospholipid chain.

(4) That the further formation of lipo-amino-acid complexes and their condensation along the phospholipid chain to form phospholipoprotein proceed spontaneously, catalysed by the proximity of the charged phosphate groups. The structure of the whole proposed 'template' is illustrated roughly in Fig. 1, although the surfaces represented as straight lines may obviously be helical in form.

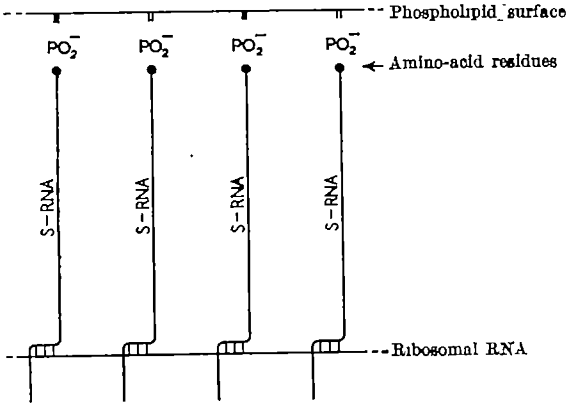

Fig. 1. Outline of structure of proposed 'template' for protein biosynthesis

In support of these suggestions, it may be pointed out that ribosomal particles prepared by detergent methods likely to remove the bulk of the lipid never seem to have activity at all comparable with, for example, pea-seedling ribosomes which apparently retain associated phospholipid[8]. Experiments *in vivo*, such as those carried out by McQuillen, Roberts and Britten[15], often seem to provide evidence that protein attached to the ribosomal RNA itself is labelled first. However, often phospholipoprotein surfaces would be destroyed by the methods of degradation used, or the material from them bound to the ribosomes themselves. The experiments of Zillig and his co-workers[16,17] in *E. coli* have indeed indicated that a lipoprotein membrane fraction (containing some RNA) is a much more active site of protein synthesis than free ribosomal particles obtained in the same experiments.

We wish to express our appreciation to Prof. J. A. V. Butler and Dr. K. S. Kirby for stimulating discussions.

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An Iron Requirement for a Dissimilatory Nitrate Reductase in *Neurospora crassa*

NITRATE reduction in *Neurospora* is usually considered to be an assimilatory process. Nitrate reductase is a flavoprotein containing molybdenum, and the sequence of electron transfer has been determined as follows:

$$\text{TPNH} \rightarrow \text{FAD} \rightarrow \text{Mo} \rightarrow \text{NO}_3^- \qquad \text{(ref. 1)}$$

where TPNH is reduced triphosphopyridine nucleotide and FAD is flavin-adenine-dinucleotide. It was shown that a deficiency of iron in the felts grown under aerobic conditions did not depress enzyme activity[2,3]. In this communication we report that *Neurospora* grown at low oxygen tensions dissimilates nitrate, and under these conditions nitrate reductase is dependent on iron as well as molybdenum for maximum activity.

Neurospora crassa, wild-type 5297*a*, was grown in a modified Fries medium[2] with sodium nitrate as the sole source of nitrogen. The following methods[4] were used to remove trace metals from the macronutrients of the culture medium: phosphate co-precipitation for iron, manganese and zinc and acid copper-sulphide co-precipitation for copper and molybdenum. The fungus was grown at 25° C., in the dark, for the prescribed period and harvested in a small Buchner funnel. The felts were well washed with 2 per cent (w/v) glucose, squeezed dry and stored at − 17° C. They were ground in a cold pestle and mortar with seven times their weight of cold 0·1 *M* phosphate

(pH 7·4) and homogenized further in a Ten Broeck glass macerater.

The enzyme was assayed as follows: 0·1–0·2 ml. homogenate, 0·1 ml. 10^{-1} *M* sodium nitrate, 0·1 ml. 2×10^{-3} *M* reduced triphosphopyridine nucleotide and 0·5 ml. 10^{-1} *M* phosphate (pH 7·4). The mixture was incubated for 10 min. at 30° C. and then 0·1 ml. 6×10^{-3} *M* *p*-benzoquinone and 2 mgm. *p*-benzoquinone reductase[5] added to oxidize residual reduced triphosphopyridine nucleotide. After 4 min. 0·1 ml. *M* zinc acetate and 3 ml. 98 per cent (v/v) ethanol were added and the precipitate centrifuged at 1,000*g* for 5 min. Nitrite was determined in an aliquot of the clear supernatant solution by adding sulphanilamide and α-naphthylethylenediamine[2]. In the control tubes, reduced triphosphopyridine nucleotide was omitted. Cytochromes *b* and *c* were viewed in a Zeiss hand spectroscope and protein determined by the Folin method[6].

Two distinct stages can be distinguished during the growth of *Neurospora* in still culture. During the first stage, 2–3 days after inoculation, the felts were submerged and growing under semi-anaerobic conditions, when nitrate was dissimilated rapidly and nitrite accumulated in the felts and in the culture medium. After 3–4 days growth, however, the felts broke the surface of the culture solution and entered a more aerobic environment, and nitrite which was produced earlier was now re-utilized. The results in Table 1 show that deficiencies of either iron or molybdenum in felts at the 2-day stage reduced enzyme activity, but after 5 days growth molybdenum deficiency only depressed nitrate reductase. The results in Fig. 1 confirm the iron requirement for nitrate reduction during early growth, when the felts were submerged, since enzyme activity of the felts increased from deficiency to sufficiency levels of iron in the culture medium. The enzyme activity of 5-day felts, however, was independent of the iron status, thus confirming previous work[2,3]. Deficiencies of either copper, manganese or zinc did not decrease enzyme activity at any stage of growth.

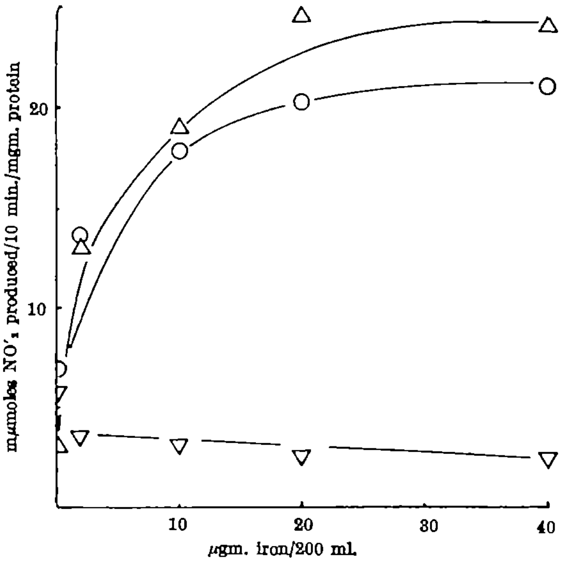

Fig. 1. Effect of the iron content of the culture medium on nitrate reductase activity in homogenates of *Neurospora crassa* harvested after 2, 3 and 5 days. *Neurospora* was grown at 25° C. in still culture depleted of iron to which 2, 10, 20 or 40 μgm./iron/200 ml. was returned. △, Felts harvested after 2 days growth; ○, felts harvested after 3 days growth; ▽, felts harvested after 5 days growth

Table 1. Effect of Deficiencies of either Copper, Iron, Molybdenum, Manganese or Zinc on the Growth of *Neurospora crassa* and on Nitrate Reductase Activity

Time of harvest (days)	Nitrate reductase and yield of the felts	Normal felts (per cent)				
		Omit copper	Omit iron	Omit manganese	Omit molybdenum	Omit zinc
2	Enzyme	100	21	98	61	103
	Yield	83	78	90	93	61
5	Enzyme	120	109	102	43	110
	Yield	49	0	67	52	8

Culture media were purified by the techniques described in the text and all the essential trace elements returned except the one to be studied. Felts were harvested after either 2 or 5 days growth in still culture at 25° C.

The effect of oxygen tension in the medium on nitrate reductase in the felts is shown in Table 2. At low oxygen tension the enzyme activity was markedly increased, nitrite accumulated and cytochromes *b* and *c* increased in the felts. The results suggest that, when oxygen is limiting, nitrate is dissimilated by an active nitrate reductase. This enzyme requires both iron and molybdenum for its activity, and in this respect it is similar to that prepared from the denitrifying bacterium, *Pseudomonas aeruginosa*[7]. During denitrification in bacteria and dissimilation of nitrate in *Escherichia coli*, the cytochrome contents of the cells increase and it is probable that they function during nitrate reduction[7-9]. A fuller account of this work is in preparation.

Table 2. Effect of Oxygen Tension in the Culture Medium on the Yield, Nitrate Reductase Activity, Nitrite and Cytochrome Contents of the Felts of *Neurospora crassa*

O_2 in N_2 (per cent)	Yield (gm. fresh weight)	Nitrate reductase (mμmoles NO_2^- 10 min./mgm. protein)	Nitrite (mμmoles NO_2^-/mgm. protein)	Cytochromes $b + c$ (relative values)
1	0·8	60·5	159	4
5	7·9	8·7	24	2
10*	9·0	2·3	9	1
21	16·8	1·3	0	1

Neurospora was grown in 500 ml. culture medium dispensed in 1½ litre Erhlenmeyer flasks. The flasks were shaken on a reciprocating shaker (100 oscillations/min.) at 25° C. and various gas mixtures of oxygen in nitrogen dispersed in the medium through a No. 2 porosity glass sinter. Felts were harvested after 3 days.

We are grateful to Dr. H. E. Davenport for determining cytochromes in the fungus. One of us (G. C. W.) is indebted to the Ministry of Agriculture, Food and Fisheries for a postgraduate studentship award.

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Synthesis of L-Methionyl-L-Glutamyl-L-Histidyl-L-Phenylalanyl-L-Arginyl-L-Tryptophyl-Glycine and its Melanocyte-stimulating and Corticotropin-releasing Activity

THE occurrence of the heptapeptide, L-methionyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine, in both adrenocorticotropins (ACTH) and melanotropins (MSH)[1-3] has led to synthesis of various peptides[4-7] related to all or part of this amino-acid sequence. In recent reports[8] the synthesis of the glutamine analogue of this heptapeptide has been described, and it was shown to possess corticotropin-releasing (CRF) activity. In the course of the synthesis of a nonadecapeptide[9] which has a sequence identical with the first 19 amino-acid residues of ACTH and which possesses ACTH and MSH activity, we have synthesized the heptapeptide in its natural form and found it to have both MSH and CRF activity.

Carbobenzoxy-N^G-tosyl-L-arginyl-L-tryptophyl glycine methyl ester[7] was decarbobenzoxylated by catalytic hydrogenation and the product was coupled with carbobenzoxy-Im-benzyl-L-histidyl-L-phenylalanine[6] by the *p*-nitrophenyl ester method[10]. The resulting protected pentapeptide (I) was obtained in the form of crystalline white powder from acetone–methanol solution: m.p. 144–146° C., with sintering from 142° C.; $[\alpha]_D^{25°}$, – 22·5° (*c*. 2, in methanol); $[\alpha]_D^{25°}$, – 25° (*c*. 1, in acetone). Analysis for $C_{57}H_{63}N_{11}O_{10}S$: Calc.: C, 62·56; H, 5·81; N, 14·08. Found: C, 62·36; H, 5·61; N, 14·16.

Peptide I was catalytically hydrogenated and the product was allowed to react with the *p*-nitrophenyl ester of carbobenzoxy-L-methionyl-γ-methyl-L-glutamic acid. The latter (peptide II) was prepared by condensation of γ-methyl-L-glutamic acid[11] with carbobenzoxy-L-methionine by means of the *p*-nitrophenyl ester method[10]. Peptide II obtained in this manner was recrystallized from 75 per cent aqueous ethanol: m.p. 134–135° C.; $[\alpha]_D^{24°}$, – 17° (*c*. 2, methanol). Analysis for $C_{19}H_{26}N_2O_7S$: Calc.: C, 53·50; H, 6·15; N, 6·57. Found: C, 53·64; H, 6·16; N, 6·86.

The crystalline protected heptapeptide di-ester [m.p. 180–182° C.; $[\alpha]_D^{25°}$, – 39° (*c*. 1, 98 per cent acetone). Analysis for $C_{68}H_{81}N_{13}O_{14}S_2$: Calc.: C, 59·67; H, 5·97; N, 12·31. Found: C, 59·44; H, 6·13; N, 13·04] was saponified and then treated with sodium in liquid ammonia[13] to remove the protecting groups. The crude heptapeptide was purified by counter-current distribution in the 2-butanol–0·5 per cent trichloracetic acid system; the material with a distribution coefficient of 0·60 was isolated and shown to be the desired heptapeptide. When acid hydrolysates of the product (tryptophan was destroyed by the acid hydrolysis) were analysed for amino-acid content by the procedure of Spackman, Stein and Moore[14], the following results were obtained: $Met_{0·8}Glu_{0·9}His_{0·7}Phe_{1·0}Arg_{1·0}$-$Gly_{1·1}$. Digestion of the synthetic heptapeptide with leucine aminopeptidase[15] gave rise to the expected constituent amino-acids as determined by quantitative analysis[16]: $Met_{0·9}Glu_{0·9}His_{0·9}Phe_{1·0}$-$Arg_{1·0}Try_{1·0}Gly_{1·0}$.

On the basis of the frog skin assay procedure[17], the heptapeptide possesses an MSH potency of 1·4 × 10^6 units per gm.; a single dose of 0·0005 mgm. caused a change in melanophore index from 1+ to 3+ within 1 hr. in hypophysectomized *Rana pipiens*, as determined by the *in vivo* assay method of Hogben and Slome[18]. When 0·1 mgm. of the synthetic product was assayed by the *in vitro* adrenal method[19], it did not exhibit ACTH activity, but 0·001 mgm. was found to cause the release of ACTH from the rat anterior pituitary, in accordance with the results of the *in vitro* procedure of Saffran and Schally[20].

It is of interest that the MSH potency of the heptapeptide is considerably higher than that reported for the glutamine analogue[8]. The question of whether or not differences in corticotropin-releasing activity exist between the heptapeptide and its glutamine analogue cannot be answered satisfactorily at the present time, since there are no standard preparations or standardized assay procedures for comparing corticotropin-releasing substances.

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Biosynthesis of ^{14}C-Sclareol and β-Sitosterol from 2-^{14}C-Mevalonic Acid

Salvia sclarea, a member of the family Labiatae, contains the bicyclic diterpene sclareol[1] and at least two acidic pentacyclic triterpenes[2]. From the non-saponifiable fraction, β-sitosterol has recently been isolated[3]; the plant thus provides a means of studying simultaneously the biosynthesis of diterpene, sterol and pentacyclic triterpene.

Several flowering sections of *S. sclarea* were cut 10–15 cm. above the root and the cut ends were immersed in an aqueous solution of 2-^{14}C-sodium mevalonate. (The 2-^{14}C-mevalonic acid was purchased from Isotopes Specialties, Inc., Burbank, California, and had a specific activity of 0·66 mc./m*M*. All carbon-14 determinations were carried out with a windowless gas-flow counter on infinitely thin samples plated and dried on nickel planchets 2·5 cm. in diameter.) A total of 60 μc. was absorbed. After standing over-night with continuous absorption of distilled water provided, the sections were divided into the various parts indicated in Table 1 and allowed to dry in air for one week. Each part (respectively) was exhaustively extracted with hot 95 per cent ethanol, which was afterwards distilled almost to dryness *in vacuo*. The residue was steam-distilled and the portion left, after drying *in vacuo*, was extracted with hot ligroin (boiling point 90–100°). The latter was washed with 1 per cent potassium hydroxide and water, then distilled to dryness. The residue contained sclareol and β-sitosterol. These were separated on active alumina, the sterol being removed with benzene, and the diterpene afterwards being removed with 2 per cent ethanol in benzene. From the benzene residue after distillation, sterol was precipitated by Sperry and Webb's method[4]. The crude sclareol fraction was sublimed twice at 10–30μ mercury pressure, at which stage sclareol melting at 99–102° was obtained. Table 1 shows the yield and carbon-14 content of sclareol and sterol expressed on a common weight basis, at what was considered a comparable stage of purification.

Table 1. INCORPORATION OF 2-^{14}C-MEVALONIC ACID INTO SCLAREOL AND β-SITOSTEROL OF *Salvia sclarea*

Plant part	Sclareol Yield (mgm./gm. dried plant)	Sclareol Carbon-14 (c./m./gm. dried plant)	Sterol Yield (mgm./gm. dried plant)	Sterol Carbon-14 (c./m./gm. dried plant)
Flowers	39·19	23,196	3·09	124,520
Flower stems	26 81	22,735	1 21	187,421
Leaves	2·75	7,276	1·36	11,238
Main stem	4 18	5,938	0·58	34,341
Stipules	23 81	35,198	1·45	96,498

On several additional sublimations and recrystallizations from aqueous methanol and ligroin, the specific activity of the sclareol (melting point 104–106°) continued to drop. On five-fold dilution with non-radioactive sclareol, hydrogenation under pressure in the presence of platinum catalyst, and two recrystallizations of the product from ligroin, dihydrosclareol (melting point 114–116°) with the following specific activities was obtained: flowers, 14 c./m. per mgm., flower stems, 9 c./m. per mgm., leaves, 12 c./m. per mgm., main stem, 7 c./m. per mgm., stipules, 14 c./m. per mgm. These figures indicate a very low order of incorporation of carbon-14 from mevalonic acid into the sclareol molecule. The sterol fractions, on the other hand, retained their high specific activity on an additional precipitation with digitonin. It is tempting to assume that, under these experimental conditions, the mevalonic acid was preferentially incorporated into the sterol molecule rather than into the diterpene. However, it is equally plausible that the turnover of the sclareol molecule may exceed that of the sterol, and other interpretations may become necessary as our information on the biosynthesis of terpenoids in higher plants is expanded. Efforts are in progress to determine in more detail the extent of incorporation of carbon-14 into the sterol (β-sitosterol) and acidic triterpene fractions.

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Utilization of the Stereoisomers of *iso*leucine by *Lactobacillus arabinosus* 17–5

IT has been reported by several authors[1-3] that *Lactobacillus arabinosus* is capable of utilizing L-allo*iso*leucine as well as L-*iso*leucine in the presence of vitamin B_6 but unable to utilize D-allo*iso*leucine, there has been only one report[4] that D-*iso*leucine was partially utilizable.

We have studied the utilization of the four stereoisomers of *iso*leucine by lactic acid bacteria such as *Streptococcus fœcalis R*, *Leuconostoc mesenteroides P*–60 and *Lactobacillus arabinosus* 17–5, which are widely used for the microbioassay of amino-acids. As a result, in contrast with the previous reports, *Lact. arabinosus* was found to be able to utilize all four stereoisomers of *iso*leucine in the presence of vitamin B_6, but not in its absence.

The medium used in the present experiment was essentially the same as described by Henderson and Snell[5], except that 0·6γ of pyridoxamine, 0·6γ of pyridoxal and 2γ of pyridoxine per 2 ml. of culture medium were used as vitamin B_6 compounds and L-isomers of amino-acids were used except for alanine, phenylalanine and serine. Incubation was carried out at 37° in test-tubes which were read at suitable intervals with a spectrophotometer at 660 mμ.

The growth-responses of the three micro-organisms to the *iso*leucine isomers in the presence or absence of vitamin B_6 are summarized in Table 1.

Table 1. GROWTH RESPONSE OF LACTIC ACID BACTERIA TO *iso*LEUCINE ISOMERS

*iso*Leucine isomers	*Lact. arabinosus*		*Str. faecalis*		*Leuc. mesenteroides*	
	$B_6(+)$	$B_6(-)$	$B_6(+)$	$B_6(-)$	$B_6(+)$	$B_6(-)$
L-*iso*Leucine	+	+	+	+	+	+
L-Allo*iso*leucine	+	−	−	−	−	−
D-*iso*Leucine	+	−	−	−	−	−
D-Allo*iso*-leucine	+	−	−	−	−	−

The concentration of *iso*leucine isomers was 2 ~ 100γ per 2 ml. tube. Incubation was carried out for 96 hr.

It is of interest that D-*iso*leucine and D-allo*iso*leucine were also utilized in the presence of vitamin B_6 by *Lact. arabinosus*. This utilization of *iso*leucine isomers by *Lact. arabinosus* was further studied in detail. Approximately the same final level of growth was attained with L-allo*iso*leucine, D-*iso*leucine and D-allo*iso*leucine as that with L-*iso*leucine at higher concentrations (Fig. 1,*A*). However, it was observed that maximum growth with L-allo*iso*leucine and D-allo*iso*leucine was attained respectively in about 24 hr. and 48 hr. longer than the time required with L-*iso*leucine. Response to D-*iso*leucine was lowest among the stereoisomers tested, and maximum growth

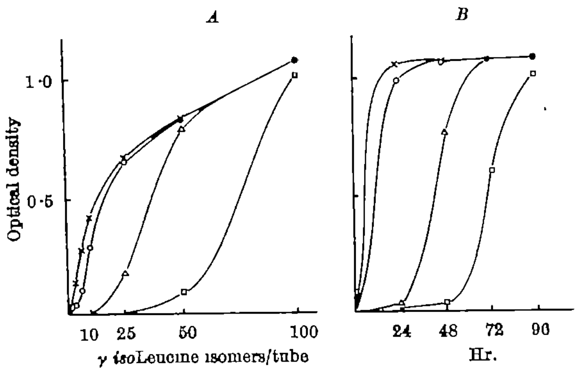

Fig. 1. Growth responses of *Lact. arabinosus* to *iso*leucine isomers. Incubation was carried out in the medium of initial pH 6 0. *A*, contained 100γ stereoisomers per tube; *B*, incubated for 96 hr. ×, L-*iso*leucine; ○, L-allo*iso*leucine; □, D-*iso*leucine; △, D-allo*iso*leucine

was reached 72 hr. later than with L-*iso*leucine (Fig. 1,*B*).

The adjustment of the initial pH of the medium to 6·0 or 5 3 resulted in more rapid growth with the stereoisomers than that of pH 6·8, as previously noted by Meister[3] in the case of L-*iso*leucine and L-allo*iso*leucine. Employment of the medium reported by Steele *et al.*[6] and the medium of Henderson and Snell[5] using mainly the DL-forms of amino-acids with modified vitamin supplement, which conformed with that of the former, resulted in slower growth response with D-*iso*leucine and D-allo*iso*leucine than that of the medium employed by us, which contained mainly L-form of amino-acids. Further work on the influence of the components of the medium revealed that the presence of excessive amounts of valine and leucine, especially the latter, inhibited the utilization of *iso*leucine isomers. This difference of the components of the media seems to account for the discrepancy between the reports on the utilization of *iso*leucine isomers. Of the vitamin B_6 compounds, pyridoxamine was most effective for the utilization of *iso*leucine isomers and pyridoxal followed. Activity of pyridoxine was lowest and was only effective for the utilization of L-allo*iso*leucine under the conditions employed by us.

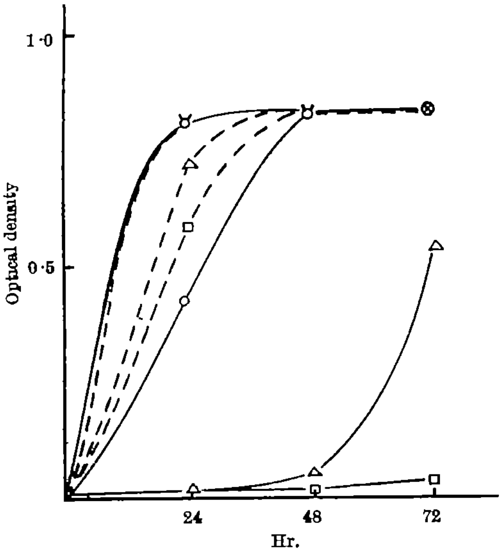

Fig. 2. Growth responses of *Lact. arabinosus* adapted for D-*iso*leucine medium and of ordinary strain to 50γ *iso*leucine isomers. ×, L-*iso*leucine; ○, L-allo*iso*leucine; □, D-*iso*leucine; △, D-allo*iso*leucine. ——, Ordinary strain; – – –, adapted strain

We also found that the strain cultured successively in the medium containing one of the other *iso*leucine isomers in place of L-*iso*leucine was able to utilize *iso*leucine isomers more rapidly than the ordinary strain cultured in the medium containing L-*iso*leucine. For example, growth responses of the strain cultured five times in succession in the medium containing D-*iso*leucine and of ordinary strain are shown in Fig. 2.

Compared with the ordinary strain, the strain adapted to D-*iso*leucine medium showed marked acceleration in response not only specifically to D-*iso*leucine but also to L-allo*iso*leucine and D-allo*iso*leucine. By employing these findings, it is possible to determine microbiologically the total amounts of synthetic *iso*leucine and allo*iso*leucine in mixtures.

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A Simple Paper Chromatographic Method for Determination of Amino-Acid Activation Profiles in Tissue Preparations

THE capacity of an enzyme preparation to activate amino-acids is usually measured by means of the amino-acid dependent isotope exchange reaction between pyrophosphate labelled with phosphorus-32 and adenosine triphosphate[1]. According to the procedure usually adopted, the labelled adenosine triphosphate is separated from the other components of the incubation mixture by treatment with charcoal. The pyrophosphate of the adsorbed adenosine triphosphate is eluted by hydrolysis with hot hydrochloric acid. The radioactivity is determined and calculated per μmole adenosine triphosphate. For measurement of whole series of individual amino-acid activating enzyme activities in tissue preparations there was a need of a simple procedure which also could be used on a semi-micro scale. A scanning method was therefore developed involving paper chromatography combined with radioautography of the papergrams.

The incubation mixture contained, in a final volume of 0·25 ml.: adenosine triphosphate–magnesium chloride (1·2 μmoles), pyrophosphate labelled with phosphorus-32 (0·8 μmole), potassium fluoride (12 μmoles), *tris*(hydroxymethyl)aminomethane buffer, pH 7·8 (30 μmoles), and the enzyme preparation (0·13 ml.). Each tube also contained 0·6 μmole of the L-amino-acid to be tested. Usually, 18 separate amino-acids were assayed. The period of incubation (35° C.) varied with the activity of the enzyme preparation from 10 to 60 min. After the incubation, 0·05 ml. of the chromatography solvent (see below) was added, giving the solution a total volume of 0·30 ml. The tubes were centrifuged, and 10 µl. of the clear supernatant was applied to Whatman No. 1 chromatographic paper. The papergrams were developed in *iso*butyric acid/ammonia/water (50 : 1 : 20) for 72 hr. at 18° C. by the descending technique. The labelled adenosine triphosphate was localized by radioautography (Gevaert, X-ray film), as well as the inorganic phosphate, which was present in the labelled pyrophosphate solution used, and which to some extent was formed during incubation. Adenosine triphosphate also was outlined by use of a 'Mineralight' lamp. For quantitative determination of specific activity, radioactivity of adenosine triphosphate was measured by a thin mica window counter (Tracerlab) on a 2·4-cm. diameter circle cut from the centre of the adenosine triphosphate spot. The radioactivity of 10 µl. of the labelled pyrophosphate solution used in the experiment, but at a concentration of 0·8 µmole in 0·30 ml., was measured in the same way. The percentage exchange values were calculated by the method of Davie *et al.*[2].

A typical radioautograph is illustrated in Fig. 1. The experiment was performed with the *p*H 5 enzyme fraction of rat liver. It is obvious from the radioautograph that the intensity of the adenosine triphosphate spots already gave a fairly good indication of the activity of activating enzymes for the different amino-acids. The general information obtained in this way may be supplemented by a more exact determination of the percentage exchange values as is shown in Table 1 using purified ribonucleoprotein particles from rat liver[3,4] and S_{50}-protein. S_{50}-protein is an enzyme preparation obtained from the *p*H-5 soluble fraction of rat liver by fractionation with ethanol and ammonium sulphate[5,6]. As is shown in Table 1, there was a slight but significant stimulation of the isotope exchange between labelled pyrophosphate and adenosine triphosphate by most of the amino-acids in the system with purified ribonucleoprotein particles. As has been shown previously[6], the capacity of amino-acid activation was rather low in the S_{50}-protein preparation, except in the cases of L-proline, L-cysteine and L-tyrosine.

Table 1. ACTIVATION OF AMINO-ACIDS BY ENZYME PREPARATIONS OF RAT LIVER, MEASURED BY ISOTOPE EXCHANGE BETWEEN PYROPHOSPHATE LABELLED WITH PHOSPHORUS-32 AND ADENOSINE TRIPHOSPHATE

Amino-acid	Percentage exchange per mgm. protein: Purified ribonucleoprotein particles	Percentage exchange per mgm. protein: S_{50}-protein
Alanine	0·4	1·3
Arginine	0 1	0·1
Aspartic acid	1·1	1 7
Cysteine	0·2	7 2
Glutamic acid	0 9	0 0
Glycine	0 5	1·2
Histidine	0 6	3 3
*iso*Leucine	0 6	1 4
Leucine	2 3	0 6
Lysine	0·9	0·4
Methionine	0·5	1·5
Phenylalanine	0·1	1 2
Proline	0 0	9·0
Serine	0 4	3 0
Threonine	0·1	3·3
Tryptophan	0·6	0·8
Tyrosine	0 8	6·0
Valine	0·2	1 7

Incubation system: adenosine triphosphate–magnesium chloride (1·2 µmoles), labelled pyrophosphate (0·8 µmole), potassium fluoride (12 µmoles), *tris*(hydroxymethyl)aminomethane buffer, *p*H 7·8 (30 µmoles), and about 2 mgm. of the enzyme protein. Each tube contained, in addition, 0·6 µmole of the L-amino-acid indicated. Final volume: 0·25 ml. Incubation for 60 min. at 35° C.

Ribonucleoprotein particles of rat liver were prepared by the method of Rendi and Hultin (ref. 3) and purified (ref. 4). Before being added to the incubation tubes they were resuspended in a medium of 0·25 *M* sucrose, 0·035 *M tris*(hydroxymethyl)aminomethane buffer, *p*H 7 8, 0·025 *M* potassium chloride and 0·01 *M* magnesium chloride. S_{50}-protein was a purified preparation of the *S*-protein of Sachs (ref. 5) obtained from the *p*H-5 soluble rat liver fraction (ref. 3).

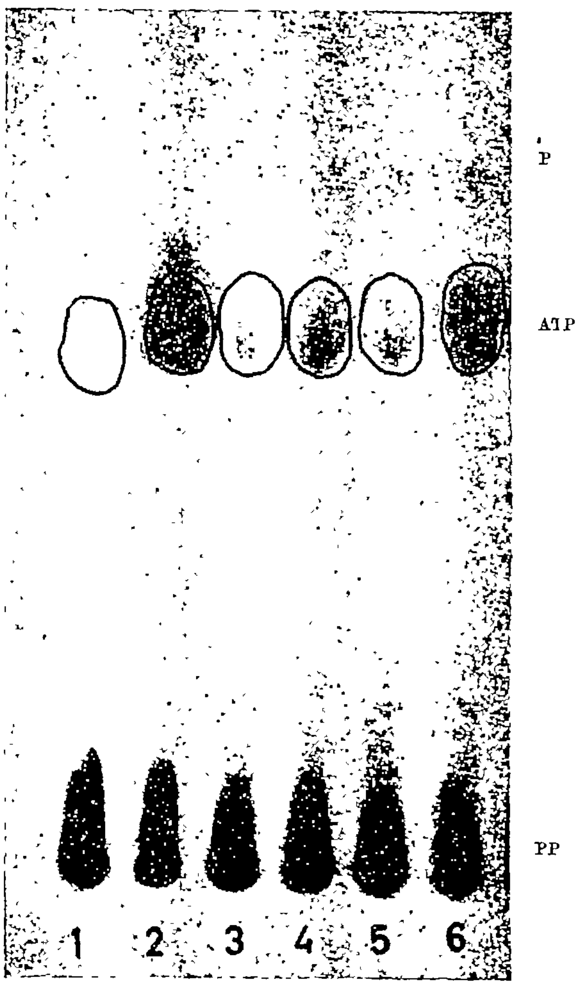

Fig. 1. Radioautograph, illustrating activation of amino-acids by the *p*H-5 enzyme fraction (ref. 2) of rat liver (0·8 mgm. protein), measured as isotope exchange between pyrophosphate-^{32}P (PP) and adenosine triphosphate (ATP). Incubation for 30 min. at 35° C. Incubation system as in Table 1. Separation of adenosine triphosphate, pyrophosphate-^{32}P and phosphate on Whatman No. 1 paper. Development for 72 hr. at 18° C., descending. Encircled areas indicate ultra-violet absorption. Samples of incubation mixtures applied on base-line contained: 1, no amino-acids; 2, amino-acid mixture of 12 amino-acids (ref. 1); 3, histidine; 4, alanine; 5, serine; 6, threonine

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PHYSIOLOGY

Anticomplementary Action of Aspirin

THE role of complement (C') in allergy[1,2], anaphylaxis[3,4], and the possible role of antigen–antibody reactions, and thus also of C'[5] in rheumatism and general fever phenomena, induced us to determine whether classical antidotes against these diseases, like aspirin (acetyl-salicylic acid)[6], salicylic acid, quinine and cortisone, have any anticomplementary action.

For this purpose the influence was studied of solutions of these agents on the hæmolysis normally provoked by the action of guinea pig's serum (C') on sheep erythrocytes (E) sensitized with rabbit anti-sheep erythrocytes serum (A) : $EAC' \to E^*$. Standard hæmolysis tubes were filled with 0·5 ml. C', diluted 1/100; 0·1 ml. E, diluted 1/50, and 0·1 ml. A, diluted 1/100, and incubated at 37° C. during 30 min. With the standard C', A and E reagents obtained from the Pasteur Institute, Paris, complete hæmolysis was always obtained in these circumstances. The dilutions were made in Michaelis's barbital buffer, $pH = 7 \cdot 4$, $\mu = 0 \cdot 15$, containing $1 \cdot 5 \times 10^{-4}$ M Ca^{++} and 5×10^{-4} M Mg^{++}. The different dilutions of aspirin, etc., tested were also made in this buffer, and the solutions thus obtained then served to dilute the C'. In this way the total volume of the reagents was kept constant, and the material to be tested for its action on C' was brought into contact with C' before any other reagent. The results are presented in Table 1.

Table 1. THE ANTICOMPLEMENTARY EFFECT OF ASPIRIN, SALICYLATE, QUININE AND CORTISONE

	Concentration (weight/volume) giving 50 per cent hæmolysis in 30 min.	Smallest concentration completely inhibiting hæmolysis in 30 min.
Aspirin	4×10^{-4}	5×10^{-4}
Sodium salicylate	no inhibition	
Quinine	$> 5 \times 10^{-4}$	slight delay of hæmolysis
Cortisone acetate	$2 \cdot 5 \times 10^{-4}$	$3 \cdot 3 \times 10^{-4}$

It is clear from Table 1 that aspirin is the only compound tested which has a strong anticomplementary action within the normal therapeutical doses, at least so far as guinea pig serum is concerned (which contains more C' than human serum). Sodium salicylate seems to have no action at all, which seems strange, as it is still much in use in many countries, instead of aspirin. Its beneficent action (if any[7]) may perhaps be explained by an acetylation during its metabolism. Quinine, which has only a slightly retarding action on C' hæmolysis, probably owes its beneficent action to a different mechanism altogether. Cortisone acetate is only anticomplementary in rather stronger than therapeutical doses : the mechanism of its action must be complex.

We have also determined which of the C' factors is inhibited by aspirin. We suspected that the inhibited factor is C_3', because of the work of Osler *et al.*[3], and we were supported in this suspicion when $EA + (C' +$ aspirin$)$, after centrifuging three times and washing with buffer (to remove the aspirin), gave rise to hæmolysis when fresh $C' +$ ethylenediamine disodium acetate (EDTA) was added[8]. This hypothesis was confirmed by using C_4' (prepared by heating the serum at 56° C. for 20 min. and then treating it with ammonium[9]), instead of C' + EDTA, because this C_3' also caused the $[EA + (C' +$ aspirin$)]$, washed three times, to hæmolyse.

We have not up to now determined whether the inhibition of C_3' by aspirin is irreversible or reversible[10]. We have been unable to determine which C' factor cortisone acetate inhibits, for, although we have chosen this form of cortisone for its relatively good water solubility, it could not be satisfactorily removed from the sheep erythrocytes by centrifuging and washing. But we have assured ourselves that the hæmolysis-preventing action of cortisone acetate is due to its action on C', and is not an aspecific protection against hæmolysis (as is the case with cholesterol[11]). Cortisone acetate, aspirin, sodium salicylate and quinine do not protect sheep erythrocytes against hæmolysis by saponin, digitonin, or dodecyl sulphate.

Work on the action of aspirin and other substances on human C' *in vitro* and *in vivo*, and on guinea pig C' *in vivo*, is still in progress ; but it can already be concluded, in the light of the results mentioned, and in view of the work of Osler *et al.*[3], that the action of aspirin on C' can quite satisfactorily explain its multiple therapeutical benefits in various pathological states such as allergy, rheumatism and fever. Thus, quantitative estimations of this type of therapeutical activity of a great many existing and new compounds may quite easily be obtained through simple complement-hæmolysis reactions.

We are indebted to the frequent and fruitful discussions we had with Dr. A. Eyquem of the Pasteur Institute, Paris.

Note added in proof. 4×10^{-4} aspirin has since proved active against much higher C' concentrations *in vitro*. This anti-complementary action, and the lack of it in the case of salicylate, has been confirmed *in vivo*.

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Possible Sources of Tartronate Deficiency in Man

SINCE exogenous tartronate (hydroxymalonate) is probably essential for the nutrition of rats[1], it has been suggested[2] that prolonged deficiency of available tartronate might result also in metabolic disturbances in man. Tartronate, which is produced only by plants[3], has chemical and physiological properties which could result in its becoming deficient in human diets. These properties are : (*a*) easy decarboxylation in hot, slightly acid solution ; (*b*) complete solubility in water; and (c) a relatively rapid rate of excretion. In the present work these sources of loss have been examined.

The loss of tartronate caused by decarboxylation may be attributed to the heating of acid foods during their preparation for consumption. To measure this effect, the homogenized samples were heated 1 hr. at 100° C., while the controls were alkalinized to *p*H 9 before the heating to prevent decarboxylation. The samples were cooled to room temperature, and the *p*H of the controls was brought back to the original value. Each was then analysed[4] for tartronic acid (Table 1). Losses were about 20 per cent for acid foods and insignificant for neutral and alkaline foods.

Table 1. LOSS OF TARTRONATE (TA) RESULTING FROM 1-HR. HEATING WITH WATER AT 100° C. OF A NUMBER OF ANIMAL AND VEGETABLE TISSUES

Tissue	Sample *p*H	Unalkalinized (mgm. TA/gm.)	Alkalinized (mgm. TA/gm.)	Loss TA (per cent)
Apple	4·0	2·78	3·27	15
Rolled oats	5·7	0·07	0·09	22
Potato	5·8	0·21	0·46	33
Corn meal	5·9	0·14	0·18	22
Tartronate	6·0	0·82	0·93	14
Rice, brown	6·3	0·25	0·30	17
Haddock	6·6	0·03	0·04	25
Milk, raw	6·7	0·25	0·27	7
Beefsteak	6·9	0·15	0·16	6
Egg yolk	9·1	0·04	0·04	0
Egg white	9·1	0·11	0·12	6

Oxidation by air during heating was eliminated as a factor, since no differences were found between evacuated and non-evacuated samples.

Discarding the water in which vegetables have been boiled results in the loss of considerable tartronate. For example, a peeled half (72 gm.) of a medium-sized potato, without further cutting, was boiled 30 min. in a covered beaker with 125 ml. of water. At the end of that time the total weight of tartronate in the potato was 5·0 mgm., while that in the water was 5·7 mgm. Thus, half the tartronate remaining after decarboxylation would have been discarded.

Volatilization with steam is relatively slight (approximately 0·06 mgm./100 gm.).

Urinary tartronate excretion was measured in serial 2-hr. samples for 12 hr. following meals containing: (*a*) about 20 mgm. and (*b*) about 650 mgm. of tartronic acid. Each meal was eaten following a 12-hr. fast. The urine samples were alkalinized to *p*H 9, evaporated to about 15 ml. and analysed for tartronic acid[4] (Table 2). The excretion-rate in this work, about 10 mgm. per 12-hr. period, was essentially the same for both meals, but represented a large fraction of the tartronate in the low-tartronate meal.

Tartronate in a stool sample was found to be about five times as concentrated as in the urine (0·028 mgm. versus 0·005 mgm. per gm.).

Table 2. URINARY EXCRETION OF TARTRONIC ACID (TA) FOLLOWING A MEAL CONTAINING: (*a*) ABOUT 20 MGM. AND (*b*) ABOUT 650 MGM. TA

Period (2 hr.)	(*a*) (mgm. TA/2 hr.)	(*b*) (mgm. TA/2 hr.)
First	1·80	3·06
Second	2·27	2·02
Third	1·44	1·80
Fourth	2·03	0·71
Fifth	0·63	1·46
Sixth	1·57	1·73
Total	9·74	10·98

Clear metabolic abnormalities are produced within two months in young rats on a tartronate-free diet, indicating that in this species, at least, exogenous tartronate may be essential to normal nutrition[1]. The first effect noted is the gradual rise in the carbohydrate-assimilatory respiratory quotients to values significantly greater than 1·00 (refs. 1, 5), accompanied by increasing obesity[1]. Minimal amounts of tartronate reduced these quotients to less than 1·00 (ref. 1). After about twelve months on the tartronate-free diet, the carbohydrate-assimilatory respiratory quotients were appreciably below 1·00 and fat formation no longer occurred[5], presumably because insulin was no longer as available for the early stages of carbohydrate catabolism. At this late stage no evident effect follows tartronate feeding. If, however, insulin is injected at the time of the test meal, high respiratory quotients are again found. A few days feeding of small amounts of tartronate can suppress this insulin effect[2]. These results and a number of low glucose tolerances suggest that at this stage an absolute or relative deficiency of insulin has supervened[2].

On the basis of results obtained with calf heart muscle, the following hypothesis has been suggested to interpret the above observations: tartronate may be a coenzyme of oxalosuccinic carboxylase in the citric acid cycle[3]; thus its deficiency in the body could result in a tendency to accumulate acetyl coenzyme *A*; much of this intermediate would be converted to fatty acids and cholesterol, requiring an abnormal amount of reduced triphosphopyridine nucleotide[6]; to supply this reduced triphosphopyridine nucleotide, an abnormal amount of glucose would have to be catabolized by the pentose phosphate route, which would require an excessive amount of insulin[7] and simulate in this respect the metabolic consequences of over-eating. If this accretion is sufficiently prolonged, disorders of the endocrine control of carbohydrate metabolism may be induced.

Whether tartronate is essential to human nutrition and, if so, whether it is possible for deficiencies to occur, are unknown, although the analogy of the obese, tartronate-deficient rat to human metabolic conditions may be significant. That losses of tartronate consistent with a mild to moderate deficiency can occur in man has been shown in this communication.

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A New Green-Leaf Growth-stimulating Factor, Phyllococosine, from Coconut Milk

KINETIN[1,2] and gibberellin[3] are known to accelerate the growth of green leaf disks floated on a medium containing one of these substances. A growth factor that stimulates leaf growth has now been discovered in coconut milk.

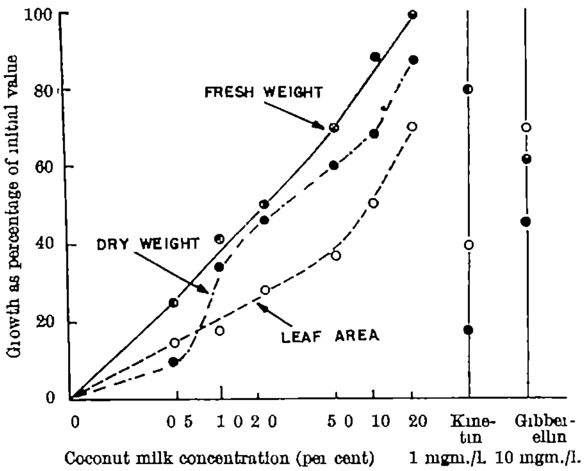

Fig. 1. Effect of coconut milk on the growth of green leaf disks. Leaf disks were incubated at 25° C. for 12 hr. under illumination of 1,000 lux. The two lines on the right show the optimum growth produced by kinetin (refs. 1 and 2) or gibberellin (ref. 3)

Raphanus sativus L. var. *acantiformis* Makino was cultured in pots in the field[1,2]. When the first foliage leaves became about 0·4–0·5 cm.^{2} in size, the pots were transferred to the dark room to increase the sensitivity of the tests, and kept there for about 24 hr. After this period, disks were punched out from the middle of the leaves with a cork borer (0·5 cm. in diameter), and each lot of fifteen disks was floated on 10 ml. of the culture solution[1,2] in Petri dishes (5 cm. diameter) and then incubated at 25° C. for 12 hr. under 1,000 lux of illumination from fluorescent tubes. The fresh weight, dry weight and leaf area of the leaf disks incubated with coconut milk were greater than those of kinetin- or gibberellin-treated disks (Fig. 1).

Growth comparisons between the media containing various concentrations of coconut milk in the presence of optimum concentrations of kinetin (1·0 mgm./l.) (ref. 1) or gibberellin (10·0 mgm./l.) (ref. 3) indicated that both gibberellin and kinetin have a synergistic effect with coconut milk (Fig. 2). When coconut milk was mixed with an equal volume of 0·1 *N* acetic acid, 0·1 *N* hydrochloric acid or 0·1 *N* sulphuric acid, and kept in the cold for 12 hr. in the dark, a considerable loss in activity resulted. The leaf-growth factor in coconut milk did not dissolve in ethyl ether, petroleum ether, benzene, chloroform or anhydrous acetone. All these facts indicate that this leaf-growth factor is different from kinetin or gibberellin.

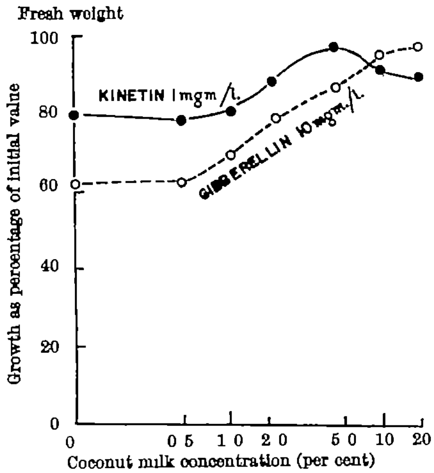

Fig. 2. Synergistic effect of coconut milk and kinetin or gibberellin Leaf disks were incubated at 25° C. for 12 hr. under illumination of 1,000 lux

The leaf-growth factor was purified according to the scheme shown in Fig. 3, and a preparation about 2,000 times more active on a dry-weight basis than the starting material was obtained.

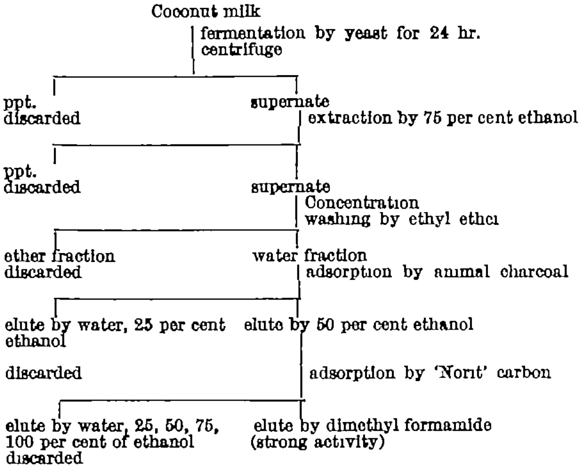
Coconut milk
fermentation by yeast for 24 hr.
centrifuge
ppt. discarded
supernate
extraction by 75 per cent ethanol
ppt. discarded
supernate
Concentration
washing by ethyl ether
ether fraction discarded
water fraction
adsorption by animal charcoal
elute by water, 25 per cent ethanol
elute by 50 per cent ethanol
discarded
adsorption by 'Norit' carbon
elute by water, 25, 50, 75, 100 per cent of ethanol discarded
elute by dimethyl formamide (strong activity)

Fig. 3. Purification of coconut milk factor

The cell-division factor from coconut milk has been extensively purified[4] and the activity has been reported to be precipitated with mercuric or lead acetate. The leaf-growth promoting factor lost its activity on treatment with these compounds (hydrogen sulphide was added to remove the excess mercuric or lead ion).

We conclude that this leaf-growth factor in coconut milk is a new growth factor and propose to name it 'phyllococosine'.

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Differential Sublimation Rates of Light and Tritium-labelled Water

WATER is an important component of the body, and the only one that can be measured directly. Body water is also useful, however, to obtain an estimate of body fat. Body water plus body density information enables one to estimate body fat with a precision of approximately 2–4 per cent when the following equation is used:

$$\text{Percentage fat} = (2{\cdot}118\,D - 0{\cdot}780\,TBW - 1{\cdot}354) \times 100$$

where D is the density of the body, and TBW is the total body water, as a proportion of body weight[1].

A valid and reliable method for measurement of total body water with a liquid scintillation spectrometer was sought in order to evaluate changes in body composition in man which occur as the result of restriction of calories and other experimental regimens. Others[1,2] have reported that tritium may be used successfully as a solute to determine total body water. The method depends on vacuum sublimation of tritium-labelled plasma to obtain suitable samples of plasma water for liquid scintillation radioassay. Since sublimation to virtual dryness requires one or more hours, it was felt that time might be saved if a shorter collection period would give the same specific activity as complete sublimation. Credence was attached to this approach because it has been observed that distillation of urine containing tritium in specially shaped vessels failed to produce a significant difference in isotope concentration in serial distillation samples[3]. When the procedure for vacuum distillation[2] was sequentially interrupted and fractional collections were obtained, it became apparent that progressive tracer enrichment of the sublimate occurred. Further, the geometry of the glassware used for sublimation altered the rate of enrichment. Although the phenomenon of tracer enrichment has long been known and has been effectively used for isotopic separation by fractionation[4], it seems important to direct attention now to this significant source of error in incomplete sublimation in view of increasing interest in determinations of body composition.

A sample of tritium-labelled water of known concentration was placed inside a Florence flask (first apparatus), which was then rotated in a dry ice and ethanol bath to freeze the sample in the shape of a thin spherical shell. The flask was connected to a vacuum system in which vapour released from the sample was collected by a trap immersed in dry ice and ethanol. The vapour trap was frequently replaced by a new one, and whatever ice had collected in each vapour trap was melted, removed, mixed, and radioassayed with a liquid scintillation spectrometer. The total procedure was repeated twice, and the normalized average results are given in Table 1*A*.

The sublimation procedure was also repeated in a second apparatus, the sample vessel of which was tubular rather than spherical. The sample was frozen in this vessel in the shape of a solid cylinder rather than a hollow sphere. With this second apparatus, the ratio of exposed area to sample volume was such that the sample melted before sublimation was completed. Later fractions were therefore condensates of the evaporated vapour from the liquid sample. The sublimation was repeated twice, and the normalized average results are given in Table 1*B*.

Table 1*A* indicates that on the average as the sublimation proceeded, each sublimate fraction contained an increasing concentration of tritium. The sample taken at the beginning of the sublimation had the lowest specific radioactivity, and the sample taken at the end of the sublimation showed the highest specific radioactivity (arbitrarily designated 100 per cent). The mean percentage difference between the first and last collection was 8 per cent.

Table 1. Results of Three Separate Sublimations (*a*, *b*, *c*) with Each of Two Apparatus Models

A, First apparatus (spherical flask)

Sublimation fraction→	1		2		3		4	
	C mμc./gm.	*N* %	*C*	*N*	*C*	*N*	*C*	*N*
Sublimation mixture *a*	9·09	93	9 20	94	9·54	98	9 73	100
b	50 2	92	52 4	96	50·0	91	54·8	100
c	50 2	91	50 5	92	—	—	55 0	100
Mean percentage		92		94		95		100
Time (min.)		10		34		51		92

B, Second apparatus (tubular flask)

Process →	Sublimation			Melting	Evaporation		
Sublimation fraction→	1	2	3	4	5	6	7
Sublimation mixture *a*	95%	95%	100%	92%	90%	95%	100%
b	86	91	100	97	93	97	95
c	96	96	95	—	96	99	100
Mean percentage	92	96	98	95	93	97	98
Time (min.)	16	32	52	68	83	107	Residue

Each value represents the mean radioassay of five aliquots of each sublimation fraction. Values are in percentages of the most radioactive sample (rounded to two significant figures). *C*, mean concentration of tritium found, in mμc. per gm. of sample; *N*, normalized mean concentration of tritium (per cent); time, mean time elapsed from the beginning of the sublimation to the termination of collection of each fraction, in minutes.

Thus, the maximum variation from the mean concentration for the entire collection was 4 per cent. Estimated total body water would therefore vary by 4 per cent if only the results from the first collection were used for the estimation.

Table 1*B* also indicates that on the average the concentration of tracer in the sublimed fractions increased with time of collection, for the first three fractions. During the collection of fraction 4, the sample melted and the liquid sample bubbled, so that the sample was physically re-mixed. The majority of the trapped specific activity from fractions five to seven was condensed from the vapour of the liquid sample, and again showed a progressive increase with time. Comparison of Tables 1*A* and *B* reveals that the variation of specific activity with time depended on the geometry of the system. Results were highly variable with the second apparatus.

In summary, one cannot predict in advance from the shape of the sample flask what the concentration of tracer in a particular fraction of the sublimate might be; but it is apparent that the concentration of tracer in a single fraction is not likely to be representative of the concentration in the total volume recovered from the sample. Errors in the sublimation are minimized by removing as much water as possible from the sample of biological fluid subjected to the sublimation procedure. Alternatively, in order to save time, a correction curve might be determined for a specific sublimation procedure, if it should be desirable to obtain only a partial sublimate for radioassay. To simplify interpretation of such a correction curve, the ratio of surface to volume of the frozen sample should be relatively large, as in a spherical flask, in order to secure reproducible results. The difference between the value of mean specific activity obtained from a correction curve and from total sublimation should be no more than 2 per cent, whereas using only the first collection from the spherical flask the variation was 4 per cent. Although it might have been predicted that an enrichment effect would have been observed both because of (1) the difference in vapour pressure of tritium-labelled water and light water at given temperature and pressure[5], (2) Graham's law, in practice this enrichment effect may or may not[3] be found, depending on the apparatus and conditions for sublimation or evaporation.

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RADIOBIOLOGY

Dose–Response Relationship in Radiation Carcinogenesis

The question whether the dose–response relationship for neoplasia induced by ionizing radiations is linear or non-linear, and with or without a threshold dose, is one of the most important problems in both basic and applied radiobiology[1].

From the experimental knowledge now available[2], and especially from the results of chromosome work of the past few years, more and more evidence indicates that in all forms of neoplasia the key step is an irreversible change in the genetic apparatus of the somatic cells, that is, a somatic mutation, consisting in cytogenetical loss caused by interference with the synthesis and functions of the deoxyribonucleic acid of the chromosomes, leading, presumably, to the deletion of growth-controlling and/or regulatory proteins as well as to the loss of tissue-specific antigens.

It has generally been tacitly assumed that this somatic mutation hypothesis for induced carcinogenesis implies a linear dose–response relationship for small doses. However, it has been pointed out (see, for example, refs. 3 and 4) that this is not correct, but that the somatic mutation hypothesis may lead to non-linear dose–response relationships when the effects are taken into account of such phenomena as indirect free-radical energy transfers, the toxicity of the carcinogenic agents which kill certain of the cells, the various adaptive mechanisms of the cells whereby they defend themselves against the carcinogenic potency of the agents, such as reunion of broken chromosomes, production of antibodies, physiological recovery factors, inter- and intra-molecular transfers of ionization-electrons and excitation energies, and so forth[5].

Thus, from a direct one-hit form of the somatic mutation hypothesis previously worked out[3,6], it follows that for radiation carcinogenesis the response R (fraction of animals showing at least one tumour among a test sample) is a function of the applied dose D (in arbitrary units) given theoretically by:

$$R = 1 - \exp\left[-\frac{k\exp[-\mu D]D}{\alpha}\right], \quad \alpha = \alpha_e + \alpha_a \quad (1)$$

Here k is the specific carcinogenicity constant measuring the carcinogenic potency of the agent for given species of test animal, genotype, phenotype, tissue and type of tumour; μ is the toxicity constant measuring the degree of toxicity of the agent; α_e is the elimination constant measuring, in the case of radiation from internally applied radioisotopes, the physical decay and metabolic elimination of the agent; α_a is the adaptation constant measuring the degree of adaptive power of the cells.

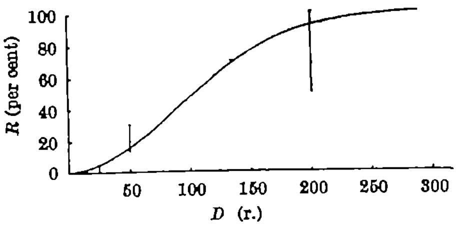

Fig. 1

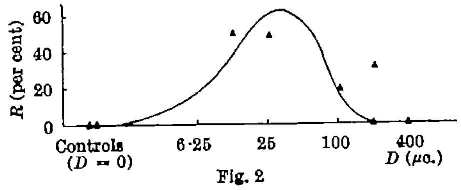

Fig. 2

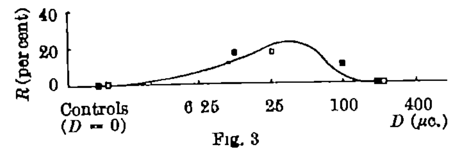

Fig. 3

Whether a threshold dose exists or not in radiation carcinogenesis cannot be decided experimentally but only on theoretical grounds, as pointed out by Mole[4]. We see from equation (1) that no threshold is required in the direct one-hit form of the somatic mutation hypothesis. However, as shown below and in Figs. 1–3, the existence of such a threshold may be simulated in certain cases by the fact that the form of equation (1) may lead to such small values of R for small doses that they are unobservable in the small samples of test animals generally used in experimental carcinogenesis.

For increasing radiation doses, the defence mechanisms of the cells may be expected to be interfered with and weakened, and thus the adaptation constant α_a will, presumably, decrease with increasing doses. If, tentatively, we make the simple assumption about this variation that α_a is inversely proportional to the dose D:

$$\alpha_a = \beta/D \quad (2)$$

we get dose–response curves which, for $\alpha_e \ll \alpha_a$, show sigmoid form, varying for small doses as D^2, and for $\alpha_e \gg \alpha_a$ are linear for small doses. Inserting (2) into (1), we can calculate the theoretical dose–response curve and compare it with experimental values by graphical fitting of the three parameters α_e/k, β/k, and μ. (The value of the parameter k can be obtained when the induction-time versus dose curve is fitted.) In Fig. 1 we show as an example $R = R(D)$ with $\alpha_e = 0$, $\mu = 0$, and $k/\beta = 6{\cdot}4 \times 10^{-5}$ fitted to the experimental values of Deringer *et al.* discussed by Mole[4]. In Figs. 2 and 3 we show as two other examples $R = R(D)$ with $\alpha_e = 0$, $\mu = 0{\cdot}06$, and $k/\beta = 6{\cdot}4 \times 10^{-3}$ and $\frac{1}{4} \times 6{\cdot}4 \times 10^{-3}$, respectively, fitted to the experimental values of Lindsay *et al.* discussed by Mole[4].

We see from Figs. 1–3 that the direct one-hit form of the somatic mutation hypothesis actually leads to a sufficiently flexible dose–response curve—without, but in some cases simulating, a threshold—which can be fitted with qualitative agreement to experimental values showing not only a linear form, but also a sigmoid form, and a form with a maximum. Thus radiation carcinogenesis does not even theoretically show one and the same form of the dose–response curve common to all cases, but the form varies from case to case, depending on the experimental conditions and the relative importance of the effects of toxicity, elimination and adaptation in each specific case.

More detailed experimental knowledge elucidating directly the toxicity effect, the elimination effect, and the adaptation effect is, therefore, necessary in each case before reliable conclusions can be drawn regarding the form of the dose–response relationship and its further application, such as for estimations of radiation hazards.

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Action of Neurohypophyseal Hormones on the Sodium Fluxes of a Freshwater Teleost

THE presence of hormones showing oxytocic and vasopressic activities in the neurohypophysis of teleosteans has been known for some time[1]. The intervention of the hypothalamo-hypophyseal complex in the regulation of the hydro-mineral balance in teleosteans has been recently proposed as a result of histological studies on neurosecretion[2]. The biological role of the hormones secreted by this complex, however, remains unknown: no antidiuretic or water-balance effect has been shown in these animals[3]. On the other hand, teleostean neurohypophyseal extracts are known to be active in certain Amphibia not only on the water metabolism[4], but also on the active sodium transport exhibited by the skin[5]. The object of the present work was to study whether, failing a clear-cut effect on water metabolism, the role of these hormones in fish is in fact concerned with sodium exchange across the gills.

The experiments were carried out on the freshwater teleost *Carassius auratus* L. The net flux, influx and outflux of sodium in a freshwater fish can be calculated by a quantitative analysis of the changes in the external medium over a period of time of the concentrations of sodium-23 and -24 added as a tracer[6]. These determinations were made over a short range of time (8 hr.) so that the radioactive outflux of the fish could be considered as negligible in the calculations. The experiments were carried out on four groups, each of six fishes (average weight of a fish, 80 gm.), placed in aerated aquaria containing 4 l. of running tap-water. The determinations of the sodium fluxes were carried out simultaneously in the four aquaria over a period of one month (March–April 1960). Each week, one of the groups acted as a control, the fishes receiving injections of a 0·9 per cent aqueous solution of sodium chloride, while the other groups received hormonal extracts. On successive weeks, different groups of fish were chosen as controls. Of the groups of fish receiving hormonal extracts, at least one was injected with acetic extract of acetonic powder of *Carassius* hypophysis (equivalent to 1 mgm. of dry powder containing 60 mU. oxytocic activity[7] per animal), or with a similar extract of *Carassius* neurohypophysis (0·1 or 0·2 mgm. powder containing 45 or 90 mU. oxytocic activity per animal). The remaining groups received either synthetic oxytocin ('Syntocinon', Sandoz) at doses of 150 mU. or 1 U. per animal, or vasopressin ('Pitressin', Parke-Davis) at doses of 400 mU. per animal.

Fishes show a marked 'shock-effect' at any disturbance, which results in an upset of their mineral balance[6,8,9], to avoid which certain precautions had to be adopted in all experiments. The fish had had 15 days adaptation in the experimental aquaria before the beginning of the measurements, and the aquaria were isolated from laboratory disturbances by a screen. The addition of radiosodium (after stopping the water circulation) and the removal of water samples every 2 hr. were carried out by remote control. Finally, the intra-muscular injections were made 17 hr. before the flux determinations.

The results are shown in Fig. 1 and Table 1. In Fig. 1, the changes with time in the external radiosodium concentration are shown on the left, and those of the external sodium-23, measured by flame-photometry, on the right. In all the experiments, these relationships were linear functions. A comparison of the regression lines corresponding to the four groups when acting as controls (black circles) with those when the groups had been treated with *Caras-*

Table 1. ACTION OF *Carassius* HYPOPHYSEAL EXTRACTS AND OF MAMMALIAN HORMONES ON THE SODIUM FLUXES OF *Carassius auratus*

	Doses	No. of determinations	Influx (μgm./hr.)	Net flux	Outflux
Controls	—	4	149 ± 33*	−13 ± 27	162 ± 27
Carassius extracts	45–90 mU. (oxytocic)	4	325 ± 7	+181 ± 19	144 ± 13
Oxytocin	150 mU.	2	290	+5	285
	1 U.	1	372	+162	210
Vasopressin	400 mU.	1	231	+87	268

* *S.E.*

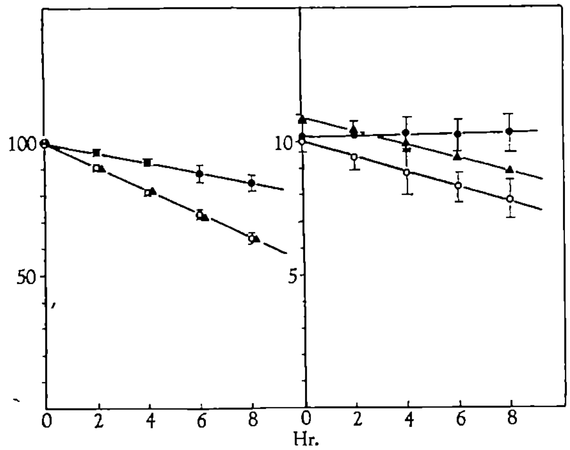

Fig. 1. Changes with time in the external media of fishes injected with neuro-hormonal extracts. Left, changes in the sodium-24 concentration (in per cent of the initial radioactivity); right, changes in the concentration of sodium-23 (μgm./ml.). Black circles, controls; white circles, after injection with *Carassius* hypophyseal extracts: black triangles, after injection with 1 U. oxytocin. The standard error of the measurements is indicated

sius extracts (white circles) shows that the slopes are significantly different for both sodium-23 and -24. In the controls, the net flux per animal is around zero: $f_{\text{net}} = -13 \pm 27$ (*S.E.*) μgm./hr., while the influx is 149 ± 33 μgm./hr. After injection with the *Carassius* extracts, the concentration of external sodium-23 falls in all the groups, indicating a positive net flux: $+181 \pm 19$ μgm./hr., while the average influx becomes 325 ± 7 μgm./hr. These increased fluxes are significantly different from the control values ($t = 5{\cdot}9$ and $P < 0{\cdot}001$ for f_{net}; $t = 5{\cdot}35$ and $P = 0{\cdot}001$ for the influx). The sodium outflux, that is $f_{\text{in}} - f_{\text{net}}$, on the other hand, remains unchanged: $f_{\text{out}} = 144 \pm 13$ in the treated fish, compared with 162 ± 27 μgm./hr. in the controls ($t = 0{\cdot}55$ and $P = 0{\cdot}6$).

The injection of oxytocin (1 U. per animal) results in an effect comparable with that obtained after injections with *Carassius* extracts. The change in external radiosodium concentration (black triangles) is identical and the slope of the linear regression line, indicating the change in concentration of external sodium-23, is similar to that obtained after hypophyseal extract injections.

Thus both *Carassius* hypophyseal and neurohypophyseal extracts and oxytocin stimulate the influx of sodium and, the outflux remaining unchanged, the result is a net uptake of sodium. The action of these hormones on the gills of teleosts is thus identical with their action on the skin of Amphibia: that is, the hormones stimulate the active influx of sodium against the concentration gradient, while the passive outflux remains unaffected.

The results given in Table 1 show that only *Carassius* extracts and oxytocin produce a clear-cut increase in sodium uptake, and that the extracts are much more active than would be expected from their actual oxytocic activity. On the other hand, vasopressin has very little activity. It would seem probable, therefore, that the neurohypophyseal principles of the fish, which are active in the mineral metabolism, are different from the mammalian neurohypophyseal hormones. The same conclusion has already been advanced by Maetz *et al.*[5] from a comparison of the activities of the hypophyseal extracts of various teleosteans with those of mammalian hormones on the active transport of sodium across frog skin ('natriferic activity'). From various investigations on the 'water-balance effect' in the frog, Heller[4], and more recently Pickering and Heller[10], Sawyer *et al.*[11], Sawyer[12], conclude that in amphibians and teleosts a 'water-balance principle' occurs, which is different from any of the mammalian hormones. Nevertheless, the biological role of the new hormone(s) would appear to be related, in any event in the teleosts, much more to the sodium than to the water exchange.

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CYTOLOGY

Implication of Chromosome Structure and Replication on Hazard of Tritiated Thymidine and the Interpretation of Data on Cell Proliferation

LABELLING of chromosomal deoxyribonucleic acid (DNA) with tritium-labelled thymidine (H^3TDR) demands consideration of the probabilities of long-term retention of the label and damage to somatic and genetic cells. These are important for the interpretation of results and for consideration of hazards associated with the use of tritiated thymidine in human beings. The model proposed by Taylor, Woods and Hughes[1] permits prediction of the persistence of the label, an implication which has not been adequately explored in previous publications by ourselves and others[2–4]. The model is based on autoradiographic observations on plant chromosomes labelled with H^3TDR during replication. In this model each chromosome is considered to consist of two halves (chromatids), both of which replicate. If H^3TDR is available during DNA synthesis for a finite period, both half-chromosomes will be labelled in their newly formed replicas. On division, all chromosomes of the daughter cells will contain the label in the newly replicated chromatid. Subsequent DNA synthesis proceeds without further availability of H^3TDR, and newly formed replicas of both labelled and unlabelled chromatids will be unlabelled.

In the daughter cells of the second division, on the average half the chromosomes will be labelled, each composed of one labelled and one unlabelled chromatid. After each subsequent division the number of labelled chromosomes per cell will be halved as the originally labelled chromatids become distributed among twice the number of cells. When the synchronously dividing progeny of a labelled cell first exceeds the chromosomal number, the fraction of labelled cells will begin to diminish, since an increasing number of cells will contain a single labelled chromatid which cannot subdivide, but must go to one daughter cell, leaving the other totally unlabelled. The expected decrease has been computed on the basis of equal distribution among daughter cells and studied experimentally in a transplantable mouse tumour by Mendelsohn[5]. The only assumption made is the non-exchangeability of DNA. This is true at the molecular level. However, exchange of part of a labelled chromatid with the corresponding portion of its unlabelled replica has been demonstrated autoradiographically in plant cells[1]. This results in redistribution of the label among a larger number of chromosomes. The extent of crossing-over in somatic and genetic cells of man is not known, but it is unlikely to approach a random distribution, as would be expected of exchangeability on a molecular level.

If one accepts this model, disregarding crossing-over for the purpose of this discussion, it may be expected that the labelled chromosomes will be randomly distributed among the daughter cells at each mitosis. Assuming that the movement of each labelled chromosome to one or the other pole of the spindle is equally likely and independent of the movement of any other chromosome, the probability of occurrence $f_k(x)$ of cells with x labelled chromosomes after k generations can be shown to follow a binomial distribution :

$$f_k(x) = \binom{n}{x}\left(\frac{1}{2^{k-1}}\right)^x\left(1 - \frac{1}{2^{k-1}}\right)^{n-x}$$

or

$$f_k(x) = \binom{n}{x}\frac{(2^{k-1} - 1)^{n-x}}{2^{(k-1)n}}$$

where n is the chromosome number. These discrete distributions have been computed for the human chromosome number 46 and are represented in Fig. 1 for the second to fifth division. In Fig. 1 the individual points $f(x)$ for each generation are joined for easier visualization. The most frequent number of labelled chromosomes per cell in successive generations is 23, 11, 5 and 2, the expected means being 23, 11·5, 5·75 and 2·87.

As can be seen from Fig. 1, about 5 per cent of cells have 0 labelled chromosomes after the fifth division. After the sixth division (not shown here), the most frequent number of labelled chromosomes is 1, and about 23 per cent of the progeny can be expected to be unlabelled. Without the influence of the random distribution of chromosomes, unlabelled cells would not appear until the seventh division. Thus, as the modal number of labelled chromosomes per cell approaches zero, the random distribution of chromosomes will favour an earlier appearance of unlabelled cells than the aforementioned decrease in the fraction of labelled cells due to the indivisibility of the originally labelled chromatids.

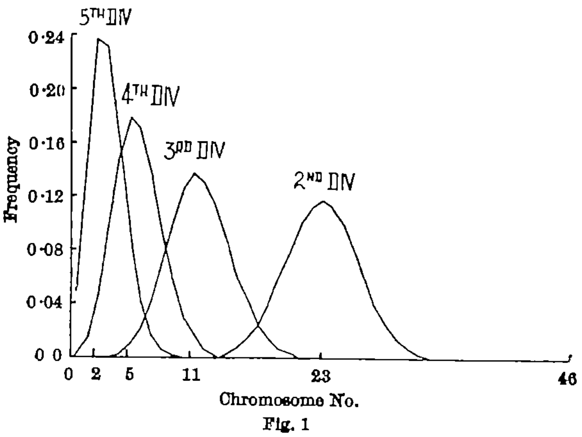

Fig. 1

Another feature of somatic cell proliferation is that a substantial portion of the progeny mature into non-proliferating cells which differentiate and die or are lost from the body, thus removing the labelled DNA.

The number of cells carrying the radioactive label in the DNA is thus continuously reduced in somatic proliferating systems such as marrow, lymphoid tissue and gastro-intestinal mucosa in three ways. There is continued loss of labelled material through death or loss of the non-dividing progeny of labelled cells ; without consideration of random distribution of chromosomes, the percentage of labelled cells is reduced continuously in an exponential manner, once the progeny exceeds the chromosomal number, because the label is not homogeneously re-mixed with all the DNA during each doubling period ; and the random distribution of labelled chromosomes among daughter cells favours an even earlier appearance of unlabelled cells among the progeny.

It has not been established that the model proposed by Taylor, Woods and Hughes pertains to mammalian chromosomes. It is now under study in tissue culture systems of human cells. If the expected agreement with the Taylor–Woods–Hughes model is confirmed, it will have a direct bearing on the interpretation of data in the study of cell proliferation, and the somatic and genetic hazard of H^3TDR in long-term work.

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Chromosome Anomaly in Two Cases of Cerebral Palsy, a Brother and a Sister

THE unsolved question of the source of many human congenital anomalies has recently received great impetus from the cytological aspect. This is largely due to an improved chromosome spreading technique which led to the establishment of the correct chromosome number in man[1]. Further evidence that a change in chromosome number was responsible for the phenotypic expression of certain clinical entities[2] stimulated the application of this

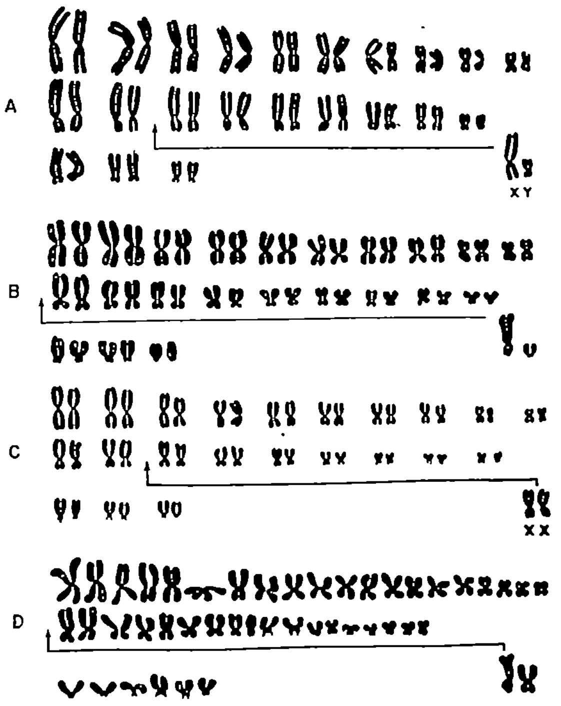

Fig. 1. *A*, Normal male; *B*, affected male; *C*, normal female; *D*, affected female. The arrows indicate the position of the *X*-chromosome in order of size and shape in relation to the centromere. The position is sixth or seventh in the ideograms of the normal children and third or fourth in the ideograms of the affected children

approach to cases of cerebral palsy of unknown or so-called developmental etiology.

In the course of our investigation of the hereditary aspect of cerebral palsy a brother and a sister came to our notice. In addition to an unusual aspastic spastic type of cerebral palsy, these children also had congenital cataract of the nuclear and posterior capsular type. After intensive investigation of this family, a rare recessive autosomal type of inheritance was reported[3]. However, recent work on metaphase plates obtained from peripheral leucocytes grown in tissue culture by a modification of the Hungerford *et al.*[4] technique showed uniform counts of 46 chromosomes in both children. Ideogram arrangements (Fig. 1) of chromosomes according to position of centromere consistently demonstrated an abnormal morphological configuration of one of the larger chromosomes with a subterminal centromere. No apparent homologous chromosome could be established for this abnormal-looking chromosome in either the boy or the girl.

Ideograms of both cases were carefully analysed and compared with those obtained from normal children of the same sex and age. Inspection of these ideograms (Fig. 1) seems to place the long unpaired subterminal chromosome in the position of the *X*-chromosome in relation to the other autosomes. Of course, the difficulty of differentiating the *X*-chromosome from some of the other elements in the complement is well recognized by cytologists.

Since both parents are phenotypically normal, a translocation on the *X*-chromosome in the mother was considered as a possible mechanism responsible for the condition in the children. The *X*-chromosome in the boy must have come from the mother, and obviously the girl also received this particular *X*-chromosome from the mother. If this theory is correct, it must be assumed that this abnormality occurred in the mother in early embryological gonadal development.

The slightly greater degree of involvement demonstrable in the boy might be due to the heterozygous sex chromosome in the male. Presumably the girl received a normal *X*-chromosome from the father and conceivably this may make a slight difference in phenotypic expression. The fact remains that there is a striking similarity in the children as to their general physical and facial appearance, their motor and cataract involvement, their mental performance, as well as the presence of the same 'abnormal' chromosome.

We thank Dr. Mizutani for technical assistance.

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BIOLOGY

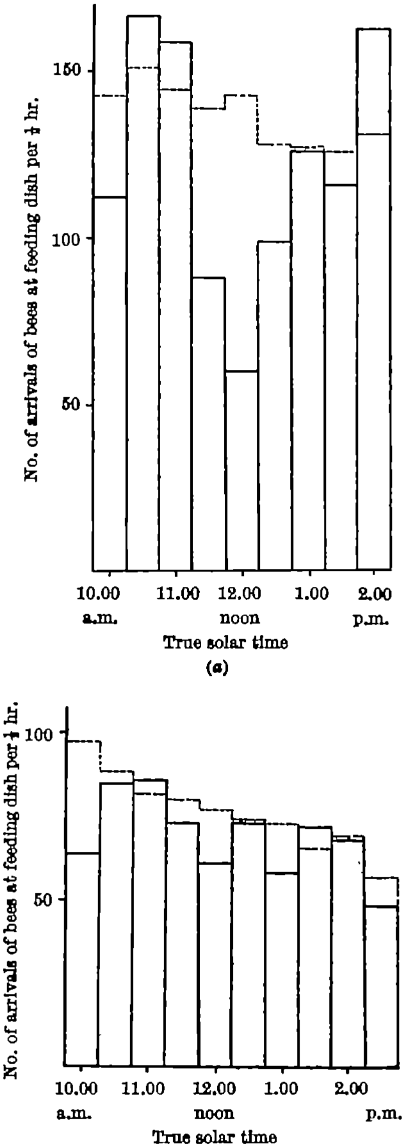
Honeybee Communication when the Sun is Close to the Zenith

THE work of von Frisch[1] and others has shown the ability of honeybees to communicate the direction of a food source by indicating the angle between the azimuths (compass direction) of the source of food and the Sun. In northern or southern latitudes, the Sun is always low enough in the sky for its azimuth to be clearly determined by the bees; but in the tropics the Sun at one or two seasons of the year passes through the zenith (that is, directly overhead), or very close to the zenith, at midday. What effect does this have on honeybee communication ?

Using facilities provided by the Zoology Department of the University College of the West Indies (lat. 18° N.), experiments to answer this question were carried out in August 1959. Bees were trained to visit a feeding dish about 200 yd. from their hive. These trained bees were marked and allowed to fly back and forth between hive and dish throughout the day. Any unmarked bees that arrived at the dish were killed. The times of arrival at the dish of marked and unmarked bees were recorded.

The combined results of six days observations (August 1, 2, 3, 10, 11, 12) are shown in Fig. 1*a*. On these days the Sun at noon was always less than 3° from the zenith. These results show a pronounced fall around midday in the frequency of arrival of unmarked bees.

The combined results of observations on August 17, 18 and 19 are shown in Fig. 1*b*. On these days the noon zenith distance of the Sun was 4·5–5·0°. There



(a)

(b)

Fig. 1. Frequency of arrival of bees at different times of day. Continuous lines indicate unmarked bees. Broken lines indicate number of visits, divided by 2, of marked (trained) bees. Combined results of days (a) when Sun at noon less than 3° from zenith; (b) when Sun at noon 4·5–5 0° from zenith

is no suggestion here of a midday fall in frequency of arrival of unmarked bees.

Bad flying conditions around midday could of course account for the fall in numbers of unmarked bees shown in Fig. 1a. But detailed weather records failed to provide evidence for such bad conditions. In any event the number of visits of marked bees does not decline at midday. Most other possible explanations, such as a daily rhythm of hive activity, or a daily rhythm in dancing behaviour of the foragers, are ruled out by the steady arrival of unmarked bees throughout the day shown in Fig. 1b.

The evidence, therefore, seems strong that honeybee communication is hindered at small zenith distances of the Sun.

Fig. 2 shows the frequency of arrival of unmarked bees calculated for different zenith distances of the Sun. For zenith distances of less than 5° the frequency was about half that at large zenith distances. Rather surprisingly, bees were still arriving steadily even when the zenith distance was less than 1°. Control experiments have ruled out the possibility that the bees arriving at small zenith distances are scout bees, and have made it very unlikely that they are bees delayed as a result of inaccurate information[2]. There seem to remain two possible explanations. Either the perception by some of the bees of the relative positions of Sun and zenith is extraordinarily acute, so that they are still able to determine accurately the azimuth of the Sun when its zenith distance is less than one or two degrees; or some method of communication is involved which is independent of the position of the Sun. Experiments are being carried out at present to examine these possibilities.

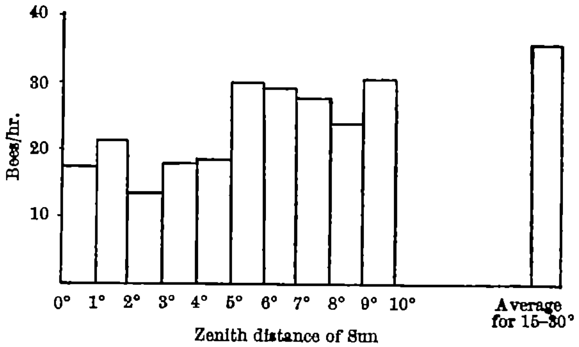


Fig. 2. Frequency of arrival of bees at different zenith distances of the Sun. Calculated from combined results of August 1, 2, 3, 10, 11, 12, 17, 18 and 19

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Morphogenetic Effects of Lipoic Acid on Amphibian Embryos

We have recently[1,2] studied the effects of mercaptoethanol (HS·CH$_2$ – CH$_2$OH) and its oxidized counterpart, dithiodiglycol, on morphogenesis in a number of different systems (amphibian eggs, regenerating tadpoles and planarians, fragments of *Acetabularia*): it was always found that mercaptoethanol inhibits morphogenesis, while dithiodiglycol has no effect or is even slightly stimulatory.

It has been recently reported by Henderson and Eakin[3] that lipoic acid (5 μgm./ml.) inhibits regeneration of the head in planarians: it therefore has the same effect as mercaptoethanol in this material. It has been suggested by Henderson and Eakin[3] that lipoic acid might act by modifying the thiol-disulphide equilibrium. It was therefore decided to compare the effects of lipoic acid and mercaptoethanol on amphibian eggs, in order to find out whether, as in planarians, the two substances act in the same way.

It was found that lipoic acid (5 μgm./ml.) and mercaptoethanol at low concentrations (*M*/300–*M*/1,000), contrary to this expectation, act in completely opposite manner on *Rana temporaria* and

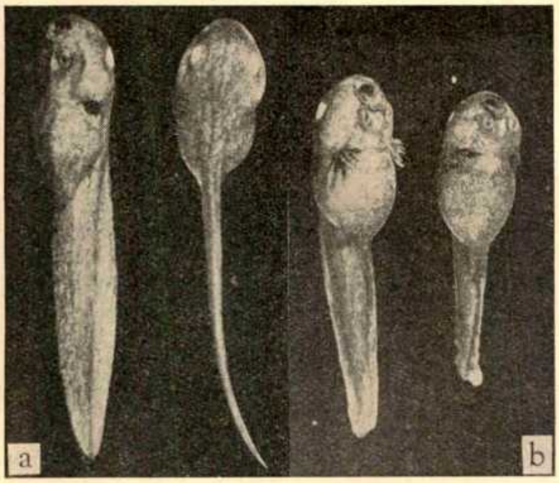

Fig. 1. (*a*) Control tadpoles of *Rana temporaria*; (*b*) embryos of *Rana temporaria* after treatment with lipoic acid (5 μgm./ml.) during 3 days

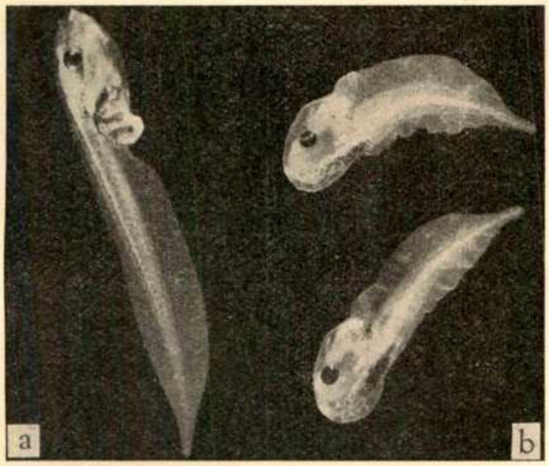

Fig. 2. (*a*) Control tadpole of *Xenopus laevis*; (*b*) embryos of *Xenopus laevis* treated during 5 days with lipoic acid (5 μgm./ml.)

Xenopus laevis embryos (gastrulæ or neurulæ): there is no delay in development, no inhibition of morphogenetic movements, and no microcephaly when these embryonic stages are treated with lipoic acid. On the other hand, this substance produces a marked inhibition of tail elongation, whereas the head and the size of the fin are essentially normal. This effect, which seems to be fairly specific of lipoid acid, is found in the frog (Fig. 1) as well as in *Xenopus* embryos (Fig. 2).

Extensive measurements of the length of the tail with an ocular micrometer showed that the tail undergoes a 25 per cent reduction in *Rana* and a 35 per cent reduction in *Xenopus* in tadpoles treated with lipoic acid.

In *Pleurodeles*, the effects of lipoic acid are very different; but, again, they are quite distinct from those observed in the case of mercaptoethanol. In this case, lipoic acid is so active in producing cell dissociation that concentrations as low as 0·3 μgm./ml. are still toxic. At still lower concentrations of lipoic acid, no consistent effect on the size of the tail can be detected. But another effect is visible, namely, an increase in pigmentation. Again, this effect is exactly the opposite to that observed for mercaptoethanol. The latter produces albinism and, in the case of *Xenopus*, the appearance of tadpoles with blue eyes and chromatophores[2]. This inhibition of pigmentation by mercaptoethanol is probably due, as shown by Roston[4], to the combination of thiols with an oxidation product of dioxyphenylalanine during pigment formation under the action of tyrosinase.

Since dithiodiglycol at low concentrations ($M/10,000$–$M/100,000$) exerts effects which are exactly the opposite to those of mercaptoethanol[1,2], it is obvious that it must act like lipoic acid; in fact, it has been repeatedly found that dithiodiglycol produces tadpoles with large heads and short bodies. It also produces rapid cell dissociation and lysis in *Pleurodeles*; an increase in pigmentation can often be noticed in this species after treatment with low concentrations of dithiodiglycol. However, tail reduction is rather exceptional in embryos treated with dithiodiglycol; it is thus possible that lipoic acid exerts a specific effect possibly related to its coenzyme activities, in the case of this morphogenetic process.

Finally, preliminary experiments have been made on the effects of lipoic acid (5 μgm./ml.) on regeneration in anucleate fragments of *Acetabularia*: a slight inhibition of cap formation, but much smaller than in the case of mercaptoethanol or cysteine, has been observed.

The present results, taken together with those of Henderson and Eakin[3], show that lipoic acid acts, according to the cell system under study, like mercaptoethanol or dithiodiglycol. This finding is in agreement with the view expressed by Henderson and Eakin that lipoic acid might play an important part in shifting or maintaining the intracellular thiol-disulphide equilibrium. Biochemical work is obviously required in order to test this hypothesis.

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Localization of the Cells of Origin of the Ventral Spino-Cerebellar Tract

The location of the cells of origin of the ventral spino-cerebellar tract has never been determined conclusively by anatomical investigations[1]. We have now systematically searched for these cells in the lumbar segments of the cat spinal cord. Cells were identified as ventral spino-cerebellar tract neurones if they were monosynaptically excited by Golgi tendon organ (I*b*) afferents and antidromically activated on stimulation of fibres in the dissected spinal half (at *Th* 13 or *L*1), contralateral to the nerve supplying I*b* excitation[2]. The position of cells encountered was derived from the co-ordinates shown by the stereotactic instrument used for insertion of the microelectrode, and histologically controlled. Fig. 1 shows the position of the 39 ventral spino-cerebellar tract neurones (five experiments) from which successful intra-cellular recordings were obtained. All these cells were activated from ipsilateral nerves and antidromically invaded from

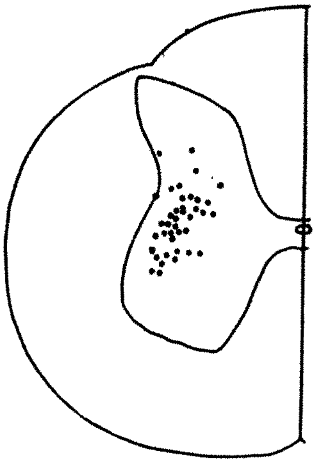

Fig. 1. Position of 39 intracellularly recorded ventral spino-cerebellar tract neurones in lumbar segments four to five

the contralateral side. Almost all units activated from the hamstring nerve were found in the upper part of the L_5 or lower part of the L_4 segment.

The present investigation has demonstrated that the ventral spino-cerebellar tract cells constitute a column of cells in the lateral part of the intermediate zone and dorso-lateral part of the ventral horn. Some cells also occur in the adjacent part of the dorsal horn. Clearly the ventral spino-cerebellar tract cells are to be distinguished from the border cells described by Cooper and Sherrington[3]. The location is, however, similar to that described by Sprague[4] for certain cells with contralaterally ascending axons, though many ventral spino-cerebellar tract cells are found more dorsally.

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A Chitin Medium for Isolation, Growth and Maintenance of Actinomycetes

SEVERAL different media have been suggested for isolation of Actinomycetes from soil[1]. Most of these contain carbon and nitrogen sources which are utilized by bacteria and moulds as well as by Actinomycetes, and therefore are not selective for the latter. Bacteriostatic and fungistatic chemicals such as phenol[2] and sodium propionate[3] have been incorporated into isolation media to suppress growth of bacteria and moulds and thus favour Actinomycetes. But such amendments at permissible concentrations frequently allow growth of contaminants and at higher levels may also suppress Actinomycetes. The report by Jeuniaux[4] that the polysaccharide chitin is widely utilized by members of the genus *Streptomyces* was confirmed in our laboratory in tests showing that all of 38 isolates of *Streptomyces* spp. utilized chitin as the sole carbon and nitrogen source, when supplied in an agar medium. In preliminary work, soil suspensions plated on this medium gave a predominance of Actinomycete colonies and very few bacterial and fungal colonies. These results suggested that a medium with chitin as the sole carbon and nitrogen source might be selective for isolation of Actinomycetes.

The medium used was similar to that of Reynolds[5] and consisted of colloidal chitin, 2·5 gm. (dry weight); dipotassium hydrogen phosphate, 0·7 gm.; potassium dihydrogen phosphate, 0·3 gm.; crystalline magnesium sulphate, 0·5 gm.; crystalline ferrous sulphate, 0·01 gm.; zinc sulphate, 0·001 gm.; agar, 20 gm. The *p*H of the medium was 7·0. Colloidal chitin was prepared by treating crude chitin alternately with *N* sodium hydroxide and *N* hydrochloric acid several times, then with ethyl alcohol until foreign matter was removed. This purified chitin was dissolved in cold concentrated hydrochloric acid, filtered through glass wool, precipitated in distilled water, and alternately sedimented and washed until neutral. Chitin-mineral agar was poured into Petri dishes over a 10-ml. base layer of water agar. A diluted soil suspension was applied to the chitin-mineral agar layer in either of two ways: (*a*) by mixing 0·2 ml. of soil suspension with 9·8 ml. of melted, cooled (42° C.) chitin-mineral agar before pouring; or (*b*) by spraying[6] 0·2 ml. of soil suspension on 10 ml. of the hardened medium in Petri dishes. Plates were incubated at 28° C. for 8 days.

Results in Table 1 show that Actinomycetes developed abundantly on chitin-mineral agar whereas bacteria and moulds were suppressed. Conspicuous zones cleared of chitin surrounded most of the Actinomycete colonies. By contrast, glucose-asparagine agar, egg albumin agar, and Czapek's agar, three commonly used media[1] which contain non-selective carbon sources, gave fewer Actinomycete colonies, more bacteria than Actinomycetes, and numerous moulds. At a lower dilution of soil (10^{-4}), the selectivity of chitin-mineral agar was still more evident. Conventional media were overgrown with bacteria and moulds which obliterated the Actinomycete colonies, whereas on chitin-mineral agar numerous Actinomycete colonies predominated. These results were verified using different soils.

Table 1. NUMBER OF ACTINOMYCETE, BACTERIAL AND MOULD COLONIES ISOLATED WHEN 0·2 ML. OF A SOIL SUSPENSION (10^{-5} DILUTION) WAS SPRAYED ON VARIOUS AGAR MEDIA

Medium	Average (5 plates) colonies per plate		
	Actinomycetes	Bacteria	Moulds
Chitin-mineral	30	0·4	0·2
Glucose-asparagine	8	14	5
Egg albumin	19	35	14
Czapek's	14	36	9

Streptomyces lavendulae and two unidentified isolates of *Streptomyces* gave more growth when cultured in chitin-mineral liquid medium (6 gm. chitin per litre) than in glucose-asparagine, egg albumin, or Czapek's broths. Chitin-mineral agar slants also supported excellent growth and sporulation of Actinomycetes.

Thus, chitin-mineral media were excellent substrates for isolation and estimation of total numbers of Actinomycetes from soil, and for growth and maintenance of cultures as well.

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Injury by Soaking of *Populus alba* Seeds

AIR-DRIED seeds of *Populus alba*, stored in closed vessels at + 5° C., are injured when submerged in distilled or tap water (see Table 1).

Table 1

Duration of soaking in distilled water	Germinated Normally (per cent)	Germinated Abnormally (per cent)	Not germinated (per cent)
0 (Control)	64·9 ± 1·95	11·8 ± 0·92	23·4 ± 1·90
15 min.	26·2 ± 0·50	40·2 ± 2·41	34·2 ± 2·41
35 min.	27·0 ± 1·20	40·0 ± 0·53	33·7 ± 1·62
60 min.	24·0 ± 0·63	45·9 ± 1·45	30·0 ± 1·33

The soaking medium was suddenly removed by suction on a Nutsch filter so that the seed-coats became almost dry. Any excess moisture of the germinating medium (filter-paper in Petri dishes) was carefully avoided. Seedlings having well-developed hypocotyl hairs showing regular growth and geotropic responses were considered as normal. (Unlike other seeds, the germination of *Populus* (and *Salix*) seeds begins with the extension of the hypocotyl, which shows negative geotropism. Soon around the end of the hypocotyl there appear 'hypocotyl hairs', very like the ordinary root hairs of other plants. These attach the plant very firmly to the substrate and play a great part in the geotropic stretching upright of the seedling. The first normal root emerges only later from the middle of the hypocotyl end. Though germination is very rapid, definitive counting was carried out on the third day after sowing, in order to recognize abnormal seedlings without doubt. Errors are indicated according to the formula:

$$\sigma = \pm\sqrt{\frac{S(x-\bar{x})^2}{n(n-1)}}$$

where x denotes the results of singular germination experiments, $\bar{x}$ their average, n the number of parallel experiments (in our case, four). Because of the small number of experiments, differences exceeding $\pm 3\sigma$ should be taken as criteria of significance.

As can be seen, soaking for 15, 35 or 60 min. equally increases the percentage of abnormal seedlings, while the decrease in germinating power is slightly significant.

Two types of abnormalities were found. Most of the abnormal seedlings show poor development of the hypocotyl hairs, absence of firm attachment to the substrate and imperfect geotropism, that is, they are leaning. On touching, they easily overturn. A few of the abnormal plants germinate inversely, that is, the cotyledons appear first. The elongation of these plants is much reduced; geotropism is completely lacking. Unfortunately, it is not possible to state quantitatively the precise proportions of these abnormalities to one another.

Table 2 provides evidence that the injuries result immediately when the seeds come into contact with water. This conclusion is not affected by the apparently poor biological value of the seeds.

Table 2

Duration of soaking in water	Germinated Normally (per cent)	Germinated Abnormally (per cent)	Not germinated (per cent)
0 (Control)	57·9 ± 2·20	14·7 ± 0·65	27·3 ± 1·81
1 min.	25·9 ± 2·32	43·6 ± 0·91	30·5 ± 2·59
30 min.	25·0 ± 1·00	41·4 ± 2·78	33·6 ± 2·10

It is certain from Table 2 that anaerobiosis cannot be the cause of this striking injury. This view is confirmed by the observation that aeration in the course of soaking or previous saturation of the water with oxygen neither prevents nor increases the injury, in contrast to the experiments of Barton[1] with beans, where the presence of oxygen was very harmful.

The injury is restricted by treatments decreasing the diffusion pressure deficit of the seeds. For example, it is entirely or partially prevented by presoaking the seeds in a 2-*M* solution of sucrose for ½ hr., and then washing it out with distilled or tap water. Moreover, the damage depends largely on the humidity of the seed, as was shown also in Yamamoto's experiments with beans[2]. At a water content of 8 per cent (on dry weight basis) the damage is very severe, but it is also evident, though slight, with seeds containing 12·6 per cent of water.

The latter facts as well as the rapidity of the effect support the view that the injury is of mechanical nature, according to Iljin's theory[3] on damage due to desiccation and rehydration: the sudden distension of the cell membrane tears the protoplasm incapable of quick swelling. This interpretation is made more convincing by the observation of Sitnikova[4] that in rapidly deteriorating seeds of Salicaceae protoplasm and membrane are connected permanently by means of plasmodesms, while in other seeds a separation takes place. This is probably in connexion with the prompt germination of most *Populus* and *Salix* species; the hypocotyl hairs most susceptible to soaking injury show a particularly high growth-rate.

The practical significance of these findings is not yet clear. I suppose that soaking injury may play a certain part in the deterioration of *Populus* seeds both in Nature and forestry, particularly if propagation is by sowing stored dry seeds. In practice, it is probably important that such damage occurs

Table 3

	Water content (dry wt. basis) (per cent)	Germinated Normally (per cent)	Germinated Abnormally (per cent)	Not germinated (per cent)
Control (sown on filter-paper withour aeration)	8·2	46·7 ± 1·2	25·0 ± 0·9	28·2 ± 0·5
Aerated with humid air for 4 hr. before sowing	14·3	64·9 ± 2·1	15·5 ± 0·5	19·5 ± 1·3

even if dry seeds are germinated on moderately moist filter paper. Aeration with humid air can 'restore' the viability of the seeds, that is, it can prevent soaking injury (Table 3)

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High Polyploidy in a New Zealand *Poa*

The basic chromosome number of *Poa* is 7. Of the New Zealand species so far examined, the majority are tetraploid ($4x = 28$) and one species is 12-ploid ($12x = 84$). A much higher number has now been found in plants of *Poa litorosa* Cheesem., collected in the Auckland Islands. Preparations of mitosis show 263–265 chromosomes (Fig. 1) and the species is therefore approximately 38-ploid. So far as we are aware, this is the highest chromosome number yet recorded in the Gramineae or in the Monocotyledons as a whole. Studies of meiosis and of the breeding system have yet to be undertaken.

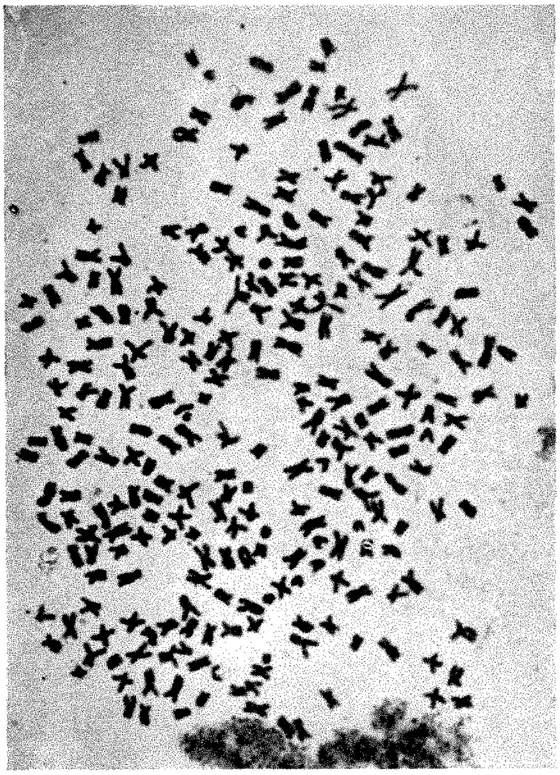

Fig. 1. *Poa litorosa*. Somatic metaphase showing 265 chromosomes (colchicine-2*BD*-Feulgen squash of root tip. × 1,500)

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BACTERIOLOGY

Absence of the Glucose Effect on the Frequency of Lysogenization in *Salmonella typhimurium*

When a sensitive bacterial cell is exposed to a temperate bacteriophage it may undergo lysis, become lysogenized, or remain uninfected. The relative frequency with which these responses occur depends on the genetic structure of the phage and the conditions of the infection. The outcome may be influenced by temperature[1], multiplicity of infection[2], and the addition of various chemicals[3,4]. In a recent paper[5], it was reported that glucose added to a nutrient agar medium affected a 100 per cent lysogenic response with *Salmonella typhimurium* strain *LT*2 ($C^{+}dg^{s}$)[6] and phage *PLT*22, while a glucose diauxie-resistant variant of this strain ($C^{+}dg^{r-1}$)[6] was found to be immune to this apparent glucose effect. These conclusions were based on observations of 'turbid' or 'less turbid' plaques as the criteria for lysogenization or lysis, respectively, of the initially infected cell. Recent attempts to duplicate these experiments were not successful, nor could any glucose effect on the frequency of lysogenization be detected using a more direct and reliable method developed by Luria and Fraser (ref. 4). (We wish to thank E. Bertani for details of the method and the indicator strain.)

This latter method involves plating cells infected with phage *PLT*22 on *EMB* galactose medium in a soft agar layer with a galactose non-fermenting (Gal^{-}) indicator strain of *LT*2. The infected cells that undergo lysis form plaques in 18 hr., infected cells that are lysogenized form dark colonies surrounded by clear halos in 48 hr., and uninfected cells form dark colonies without halos in 48 hr.

The maintenance of strains, *Salmonella typhimurium LT*2 ($C^{+}dg^{s}$), the glucose-resistant variant ($C^{+}dg^{r-1}$), and the Gal^{-} variant indicator (which was also methionine-negative and histidine-negative), and the production and maintenance of phage were as previously described[6].

In the first two experiments the infection procedure was essentially as described by Bertani[4]. An aerated overnight culture of strain *LT*2 ($C^{+}dg^{s}$), grown in nutrient broth, was diluted into fresh nutrient broth and aerated at 37° C. to about 5×10^{7} cells/ml. The

Table 1. Frequency of Lysogenization with Glucose present during the Early Phase of the Latent Period

Media during first 10 min. of latent period	Lytic centres*	Lysogenic cells*	Uninfected cells*	Frequency of lysogenization (per cent)
Nutrient broth	35	34	36	49
Nutrient broth plus 0·2 per cent glucose	51	38	27	43
Nutrient broth plus 2·0 per cent glucose	50	41	34	45

* Figures represent average of three plates (0·1 ml. per plate)

Table 2. Frequency of Lysogenization with Glucose present during Adsorption and during the Early Phase of the Latent Period

Media during adsorption and first 10 min. of latent period	Lytic centres*	Lysogenic cells*	Uninfected cells*	Frequency of lysogenization (per cent)
Nutrient broth	30	40	30	57
Nutrient broth plus 0·2 per cent glucose	45	33	20	44

* Figures represent average of three plates (0·1 ml. per plate).

Table 3. FREQUENCY OF LYSOGENIZATION WITH GLUCOSE PRESENT DURING ADSORPTION AND DURING THE ENTIRE LATENT PERIOD

Media during adsorption and entire latent period	No. of tubes plated	Average No. of cells per tube	Total lytic centres	Total lysogenic cells	Total uninfected cells	Frequency of lysogenization (per cent)
Nutrient broth	30	2·5	25	26	24	51
Nutrient broth	30	2·0	20	16	24	44
Nutrient broth plus 1·0 per cent glucose	30	1·7	19	14	17	42
Nutrient broth plus 1·0 per cent glucose	30	1·7	22	16	15	42

cells were centrifuged, washed in buffered saline, recentrifuged, and re-suspended in buffered saline to about $2·3 \times 10^{8}$ cells/ml. Phage *PLT*22 was then added at a multiplicity of 1 and adsorption was allowed to take place for 10 min. at 37° C. in either non-nutrient media or nutrient media, with or without glucose as indicated. The infected culture was then diluted 10^{-3} into nutrient broth or nutrient broth plus glucose, and aerated at 37° C. for 10 min. At this time they were diluted again into homologous media to about 1×10^{3} cells/ml., and plated in triplicate on *EMB* galactose plates. Plating was done before the first bursts, which occurred about 35–40 min. after infection. Assays for free phage showed that after 10 min. more than 95 per cent of the phage had been adsorbed to the bacteria.

When cells of *LT*2 were infected with phage *PLT*22, either in non-nutrient media or in nutrient broth, and afterwards incubated in nutrient broth, about 50 per cent of the cells became lysogenic. When the infected culture ($C^{+}dg^{s}$) was exposed to 0·2 per cent or 2·0 per cent glucose for 10 min. after the 10 min. adsorption in non-nutrient media without glucose, no increase was observed in the frequency of lysogenization (Table 1). In a similar experiment the cells were infected as above, except that adsorption was allowed to take place in either nutrient broth or nutrient broth plus 2·0 per cent glucose. As before, glucose did not increase the frequency of lysogenization (Table 2).

The following experiment was performed to determine the frequency of lysogenization in cells exposed to glucose during adsorption and for the entire latent period. Log phase cultures ($C^{+}dg^{s}$) with about 1×10^{8} cells/ml. were infected in nutrient broth or in nutrient broth plus 1·0 per cent glucose with phage at a multiplicity of 1 and adsorption allowed to take place for 10 min. with aeration at 37° C. The cultures were then diluted to about 15–20 cells/ml. and 0·1 ml. samples were distributed to 30 small test-tubes and kept in a 37° C. water-bath for the entire latent period. (The latent period in nutrient broth plus 1·0 per cent glucose was found in a single-step growth experiment[7] to end in 70 min.) The dilution was made in order to prevent re-infection from early bursts, since the cells were not plated until they had been incubated for 70 min., and also to allow the detection of haloed colonies in the presence of plaques. The contents of the tubes were then plated with the *Gal*$^{-}$ indicator strain on *EMB* galactose plates as previously described. Tubes were scored according to which of the following appeared on the indicator plate: plaques (lytic response), dark colonies with halos (lysogenic response), or dark colonies without halos (sensitive response). In this experiment as well, there was no increase in the frequency of lysogenization due to the presence of glucose (Table 3).

Glucose has been shown to inhibit inducible enzymes concerned 'primarily with catabolism' by somehow augmenting repression of enzyme formation in the presence of the inducer[8]. The absence of the glucose effect on the decision between the lytic and lysogenic response by temperate phage *PLT*22 indicates that such inducible enzyme formation is probably not a factor in phage development under the conditions employed in these experiments. However, since glucose has no apparent effect on the synthesis of enzymes concerned with biosynthetic processes, enzymes the syntheses of which are no doubt controlled by repressor effects[9,10], the lack of effect of glucose on the frequency of lysogenization should not be interpreted to mean that the protein synthesis required for the lytic response[4] is not under repressor control.

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Isolation and Some Properties of the Phages of *B. cereus* NRRL 569

So far not enough is known about the isolation and virulent phages of *B. cereus*. Kellenberger[1] studied the morphology of the temperate phages of a lysogenic *cereus* strain. McCloy's phages isolated from the lysogenic *B. cereus W* strain[2] showed anthrax specificity. The phages of Pollock's *B. cereus* 569 strain was not known until recently[3].

Isolation of the phages of *B. cereus* 569 was attempted from five samples of soil, in each case successfully. The selective enrichment was performed as follows. The vegetative cells of the streptomycin-resistant *B. cereus* 569 were added to the samples of soil suspended in *YP* (ref. 4) liquid media with 25 γ/ml. streptomycin. This culture was incubated for 4 hr. with vigorous shaking. Streptomycin suppressed the growth of other contaminating bacteria. The enriched phage suspension was propagated further by the usual methods.

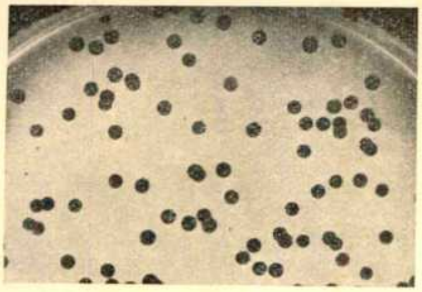

Fig. 1. Large, clear plaques of the virulent phages of *B. cereus* 569

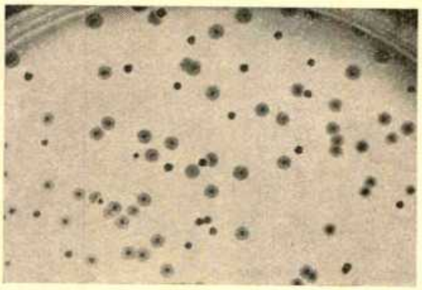

Fig. 2. Irregular clear and target-like plaques of the virulent phages of *B. cereus* 569

The propagation of the phages of *B. cereus* 569 was successful only in the *YP* (ref. 4) media. The common meat extract and Pollock's widely used media[5] were not useful for this purpose. However, with these media after being completed with Ca^{2+} (M/600) or Mn^{2+}, our phages caused the lysis of the cells of *B. cereus* 569.

On the basis of plaque morphology, our phages could be separated into three different groups: (1) clear; (2) target-like; (3) turbid plaques. According to the shape and form of the plaques we succeeded in differentiating some types in each group. The turbid plaques did not give a pure line in the subcultures; the clear and target-like mutants appeared in 10 per cent.

The above-mentioned basic types are represented in Figs. 1 and 2.

The results of the host-range experiments may be summarized as follows. The isolated phages are species specific. They do not lyse anthrax and other Gram-positive bacilli (*megaterium*, *subtilis*) and cocci. The results of the investigations on many different *cereus* strains show that a group of the *cereus* bacilli is not sensitive to the phages isolated on the strain 569. The strains did not lyse nor did they absorb the phages the autolysate of which[6] produced precipitation with the anthrax polysaccharide antibody. The *B. cereus* strains the autolysate of which did not precipitate the above-mentioned antibody were sensitive to phage. On the basis of the phage sensitivity the group of the *cereus* bacteria, considered as a uniform one, may be divided into two groups: (1) sensitive to the phages of *B. cereus* 569; (2) resistant to them.

There is an interesting correlation between the phage sensitivity and structure of the cell wall. The results of earlier experiments[6] showed that the *cereus* cell wall polysaccharide which precipitates the anthrax antibody consists of galactose and glucosamine; the non-precipitating one is made up of glucose and glucosamine with another unknown hexosamine. The present experiments suggest that the isolated phages affect those *cereus* bacilli the cell-wall structure of which agrees with those of *B. cereus* 569, showing that the polysaccharide of the cell wall consists of glucose, glucosamine and another hexosamine.

Further detailed characterization of our phages and the other experiments will be published elsewhere.

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GENETICS

Diurnal Temperature Fluctuation and Genetic Variance in *Drosophila* Populations

Four populations of *Drosophila pseudo-obscura* were started from a common founder stock in November 1956, by Dr. L. Levine and me. Since that time, two of them, K 1 and K 2, have been maintained under conditions of temperature which, while slightly differing from year to year, have always been constant diurnally. Concurrently, the populations V 1 and V 2 have been kept in diurnally fluctuating conditions with again slight difference of mean from year to year, but always: (1) with the same mean temperature as the K populations and (2) undergoing a regular 10° C. oscillation of temperature during each period of 24 hr. A more detailed account of this type of environment will be found in a recent publication[1]. All populations were initially polymorphic for the two gene-arrangements Arrowhead and Chiricahua ($q_0 = 0·5$), of the third chromosome, and all populations are now largely, if not completely, monomorphic[2]. The changes in the polymorphic system which appear to be similar in both K and V populations will be discussed elsewhere (Beardmore, J. A., and Levine, L., in preparation).

Additive genetic variance in each of the four populations was estimated in the following way. Virgin flies were collected and mated assortatively for the number of chætæ on the fifth sternite. Two samples of five flies of each sex were drawn from each culture and the chætæ on the fifth sternite of each fly were counted. These counts permitted the regression for chæta number of offspring on mid-parent to be calculated for each sex in each population. The slope of the best-fitting regression for the V populations ($b = 0·342$) is considerably steeper than that calculated for the K populations ($b = 0·215$) and the significance of this difference closely approaches the 1 per cent level. Table 1 gives the analysis of variance.

The estimates of the regression coefficients are also estimates of h^2, the heritability of the character and indirectly, therefore, of the additive genetic variance (σ_g^2) of the populations for this character since $h^2 = \sigma^2_g/\sigma^2_p$. The variance of parental chæta number

Table 1. ANALYSIS OF VARIANCE OF REGRESSION OF FIFTH STERNITE CHÆTA NUMBER OF OFFSPRING ON MID-PARENT

Source	Mean square	d.f.	t	P
Differences between means	1·63	7		
Overall regression	121·60	1	11·17	very small
Differences between regression slopes				
(i) between K and V	6·48	1	2·574	$\simeq 0·01$
(ii) within K and within V	1·01	6		
Residual	0·978	624		
		639		

(σ^2_p) in the K populations is not significantly different from that of the V populations ($F = 1·09$, $P > 0·2$). The ratio of the heritabilities of K and V populations may, therefore, fairly be taken to represent the ratio of the additive genetic variance present in the populations.

Thoday[3,4] has considered the way in which living organisms may accommodate themselves to environmental fluctuation. His thesis is that long-term oscillations of the environment will be met by genetic flexibility of populations. Such variation is thought of as having a period several or many times the generation length of the organism. Oscillations of a short-term nature (or less than the generation time of the organism) will be accommodated by developmental flexibility. The results reported here show that a regular environmental fluctuation with a period of oscillation equal to about 5 per cent of the generation time of the organism is highly effective in promoting the maintenance of additive genetic variance by populations.

It is true that what applies to genetic variance for bristle number may not apply to other characters, and it is also true that there may be reasons peculiar to *Drosophila* which render a result of this kind less surprising than it would be in some other organisms. Nevertheless, it is quite clear that the greater genetic variance in the V populations is a direct result of the diurnal environmental variation. Two conclusions follow: (1) that the greater developmental flexibility[3] or homeostasis[5] shown by the V populations for which we have additional evidence (Beardmore, J. A., and Levine, L., in preparation) may be dependent on greater heterozygosity; (2) the probability of evolutionary survival of V-type populations is greater than that of K-type populations.

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FOREST PRODUCTS

A Method of obtaining Uniform Distribution of Wood Preservatives in Toxicity Test Blocks

THE standard method[1] of measuring the toxicity of wood preservatives consists of exposing small wood blocks, containing a range of loadings of the preservative, to attack by a specific wood-destroying fungus or insect and noting the loading required to inhibit attack. The blocks are prepared by impregnating them completely with different strength solutions of the preservative in an appropriate volatile solvent which is afterwards allowed to evaporate off. The average concentration of preservative substance left in each block is obtained from the weight of solution absorbed.

Some time ago we showed that during evaporation of the solvent the preservative tends to concentrate near the surface so that its final distribution in the block is uneven[2]. This happens with all types of preservative substances, whether in aqueous or organic solvent solution, and probably the only exceptions are certain water-borne formulations which are designed to react and become insoluble soon after impregnation. The outward movement of solutions during the drying of porous media and the resultant migration of the solute to the surface are quite well known, and have been observed with a number of other materials such as paper, textiles and soil.

Because of this uneven distribution the concentration of preservative to which the test organism is exposed is not precisely known, and this introduces some uncertainty about the accuracy of the toxic limits found. This has been recognized for some time[3,4], but no practicable method of ensuring a more even distribution has so far been available.

Recently we have found that a comparatively uniform distribution of various types of preservative can be obtained by cooling the blocks below the

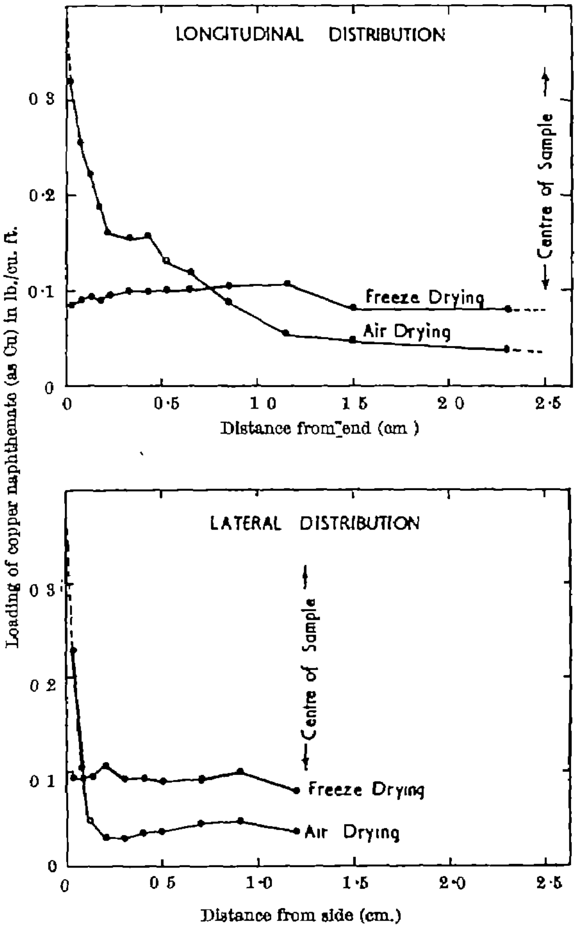

Fig. 1

freezing point of the solution directly after impregnation and subliming off the solvent under vacuum.

Fig. 1 shows the distribution in a standard-size beech block 5 × 2·5 × 1·5 cm., impregnated with a 5 per cent solution of copper naphthenate in benzene, after removal of the solvent by this method, compared with that in a similarly impregnated block dried under atmospheric conditions in the normal way.

Work on this method is still in progress, including its use in the preparation of uniformly loaded samples of the larger size used in field service tests on preservatives, for which purpose this investigation was initially undertaken, and a more detailed report will be published later.

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SOIL SCIENCE

Soil Phosphate Solubility

The inorganic phosphate content of agricultural soils is very low (about 0·02 per cent phosphorus), and attempts to determine the nature of the phosphate compounds are beset with many difficulties. X-ray and optical methods cannot be used without first concentrating the phosphate fractions, and the process of concentration generally brings about changes of phosphate form. Methods based on chemical extractants effect a more or less complete redistribution of the soil phosphate compounds during the actual extraction and, in any event, do not act specifically on single phosphate components. Recent approaches to the problems have used solubility methods, in which attempts have been made to relate soil phosphate solubility of intact soil to the solubilities of known pure phosphate compounds. Based on experimental figures for the solubility products of pure phosphates, criteria have been proposed for assessing the existence of basic calcium phosphates, and iron and aluminium phosphates in soils[1]. However, the application of these criteria to experimental determinations of soil phosphate solubility[2] has not been wholly successful[3]. Thus, while the results for some soils obey the relationships for pure compounds, others do not[3], and the proposition that these can be attributed to substances of intermediate composition is not wholly satisfactory.

In the case of basic calcium phosphates, which are generally assumed to be the main phosphate forms in non-acid soils, the solubility relationships are conveniently described in terms of the 'phosphate potentials' and 'lime potentials' of aqueous solutions in equilibrium with the solid phosphates. The potentials are defined by the equations:

$$\text{Phosphate potential} = \tfrac{1}{2}p\text{Ca} + p\text{H}_2\text{PO}_4$$
$$\text{Lime potential} = p\text{H} - \tfrac{1}{2}p\text{Ca}$$
$$\text{where } pX = -\log_{10}(\text{activity of the ion } X)$$

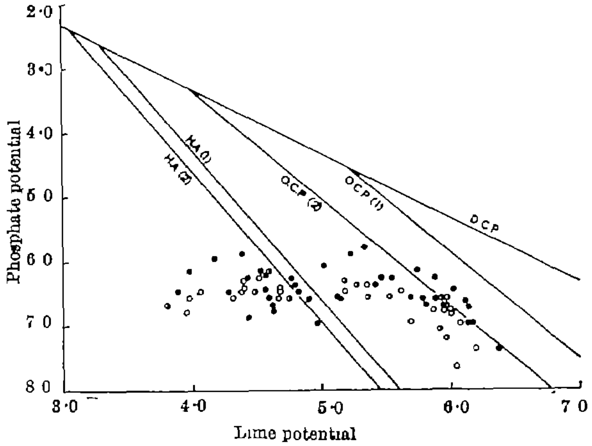

Fig. 1. Plot of phosphate and lime potentials of soils in relation to those of pure calcium phosphates. ●, Field application of 1,000 lb. phosphate (P_2O_5) per acre; ◑, of 500 lb. per acre, ○, of 0 lb. per acre. *D.C.P.*, dicalcium phosphate; *O.C.P.*, octocalcium phosphate from supersaturation (1), from undersaturation (2); *H.A.*, hydroxyapatite from supersaturation (1), from undersaturation (2)

For a pure calcium phosphate there is a linear relationship between the phosphate potential and the lime potential, the slope of which is governed by the chemical composition of the compound and the intercept of which is found from the experimentally determined solubility product. The linear relationships shown in Fig. 1 for dicalcium phosphate, hydroxyapatite and octocalcium phosphate are those used by Aslyng[2], based on the solubility products of Bjerrum[4]. The pairs of lines for hydroxyapatite and octocalcium phosphate are based on the solubility products determined by approaching equilibrium from undersaturation and supersaturation. If, then, one of these compounds exists in a soil, the experimentally determined phosphate and lime potentials of the soil should fall on the line for that particular compound.

To test this, the phosphate and lime potentials of soils enriched in the field with 0, 500, 1,000 lb. phosphate (P_2O_5) per acre as superphosphate at twenty-three sites in Britain were measured by a method similar to that described by Aslyng[2]. The soils, at soil/solution ratios of 1 : 10, 1 : 5, 2 : 5, and 4 : 5, were shaken for 16 hr. with 0·01 *M* calcium chloride solution. Each soil/solution ratio was replicated three times. The *p*H values of the suspensions were measured with a glass electrode, and, after centrifuging, the phosphate concentrations of the extracts were determined by the method of Lucena-Conde and Prat[5]. The activities of the Ca^{++} and $H_2PO_4^-$ ions were calculated from the analytical figures using the known dissociation constants of phosphoric acid[2] and the first approximation to the Debye–Huckel theory. The potentials for the soil/solution ratio of 2 : 5 were calculated and plotted in Fig. 1.

Although some of the points fall near the lines for the pure compounds, the overall distribution suggests either that no definite forms of calcium phosphate can be inferred to exist in soils or that the apparent solubility relationships are being influenced by other factors. That the latter is likely is seen from the fact that all soils of low *p*H (low lime potential) have solubilities lower than that of hydroxyapatite, whereas soils of high *p*H have solubilities greater than that of hydroxyapatite. This is shown more strikingly by

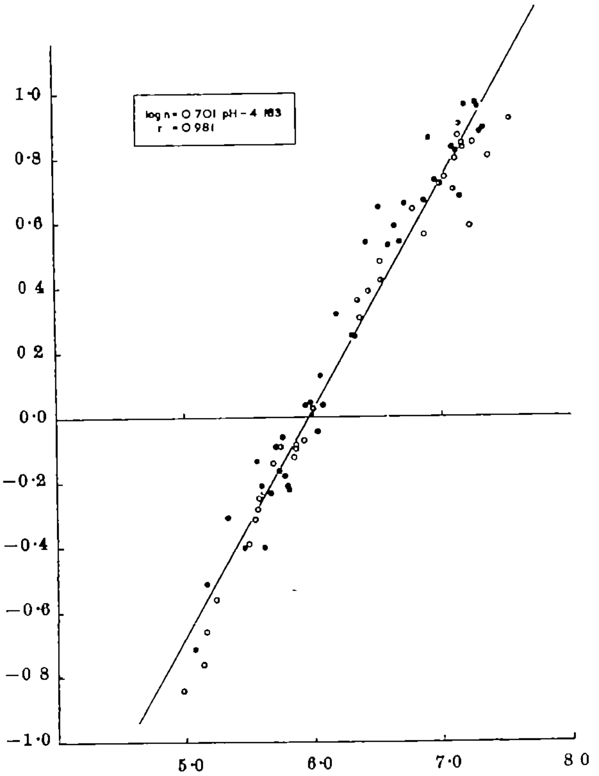

Fig. 2. Plot of logarithm of saturation index of hydroxyapatite for the soils as a function of soil pH. Symbols as for Fig. 1

calculating the apparent ionic product (I) of hydroxyapatite in the soil suspensions and relating this to the true solubility product (L) in pure systems. A 'saturation index' n can be defined (Bjerrum, quoted by Schmidt-Nielsen[6]) as:

$$n = (I/L)^{1/9}$$
$$\text{or } \log n = (pL - pI)/9$$

The factor 1/9 appears because the solubility product of hydroxyapatite, $Ca_5(PO_4)_3OH$, is a product of nine ion activities. Values of log n for the soil systems are plotted as a function of pH in Fig. 2. There is a consistent linear relationship with the regression equation:

$$\log n = 0{\cdot}701\, p\text{H} - 4{\cdot}183$$

and a correlation coefficient of 0·981 ($P < 0{\cdot}001$).

All soils with pH values above 6·0 have a phosphate solubility greater than that of hydroxyapatite, and, since hydroxyapatite is stable in this pH range, it should theoretically be precipitated. Why it does not has yet to be elucidated, but the results for soils resemble closely an effect observed by Greenwald[7] and Ericsson[8] for pure hydroxyapatite in the presence of calcium carbonate and for dental apatite which always contains a little calcium carbonate. These authors found that, in absence of carbonate ions, the solubility of hydroxyapatite obeyed the solubility product principle but that, in presence of carbonate ions, the principle was apparently disobeyed. Ericsson found that, in the presence of carbonate ions, the saturation index n varied with pH according to the equation:

$$\log n = 0{\cdot}44\, p\text{H} - 2{\cdot}23$$

Greenwald attributed the increase of solubility in the presence of carbonate ions at high pH to the formation of complex ions in solution, but it is difficult on this basis to account for the decrease in solubility found by Ericsson at low pH. Ericsson's regression equation differs from that found for soils of which carbonate ions are also normal constituents. Other anions, such as silicate, present in soils could have an effect on apatite solubility similar to that of carbonate, and this may account for the difference in the two regression equations. Nevertheless, that similar relationships seem to hold is both interesting and important.

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Direct Methods of Estimation in the Spectrographic Analysis of Cobalt in Soil Concentrates

THE relationship which forms the basis of the internal standard method of spectrographic analysis may be written:

$$\log I_T/I_S = n_T \log C_T + \log a_T/a_S C_S^{n_S}$$

where I_T, I_S are the respective intensities of the analysis and internal standard lines; C_T, C_S are the respective concentrations of the analysis and internal standard elements; and a, n are constants dependent on the general conditions of excitation. In its application to the determination of trace constituents in biological concentrates[1], the internal standard, which is iron, varies in content, and the calibration involves three steps: (*a*) obtaining plots of log I_T/I_S (background-corrected) against log C_T for sets of standard mixtures K, L, M, N, . . . containing increments of the internal standard element from one set to the next; (*b*) selecting one of these as the standard working curve, for example, the plot from the M set; and (*c*) deriving a correction graph for the C_S value of the sample. The calibration curves for cobalt, namely, log I_T/I_S versus log C_T (10–1,000 p.p.m.) and log $I_S/I_{S(M)}$ versus log C_S (2·0–50·0 per cent Fe_2O_3), are obtained as straight lines of unit gradient.

In soil analysis the concentration procedure adopted[2] is to precipitate the trace constituents on a carrier precipitate of aluminium and iron oxinates, which on ignition yields essentially a residue of aluminium oxide in admixture with iron oxide (Fe_2O_3) and the trace elements. In the treatment of an acetic acid soil extract[3], a solution of iron in hydrochloric acid (equivalent to 2·0 mgm. Fe_2O_3) is added prior to the concentration, since with the quantities involved, acetic acid extracts only traces of iron (c. 0·1–0·5 mgm., ref. 3), although occasionally amounts up to the order of 1 4 mgm. (equivalent to 2·0 mgm. Fe_2O_3) may be extracted. The final computation of results therefore requires (i) photometric evaluation of the sample spectrum for log I_T/I_S, (ii) analysis of the concentrate for C_S, (iii) correction of log I_T/I_S for the C_S value of the sample, (iv) determination of C_T for the corrected log I_T/I_S value via the standard working curve, and (v) evaluation of the concentration in the original material on dividing C_T by the appropriate factor.

A modification of the above procedure involves adding to the residue, after weighing (w_1 mgm.) but prior to mixing, 30 mgm. purified Fe_2O_3. The whole is then mixed in an agate mortar and proceeded with in the usual manner. The percentage internal standard may be estimated directly as $100 \times \left[\frac{32}{w_1 + 30}\right]$, that is, $100 \times 32/w$, where w is weight of final concentrate. The error contained in this assumption may be computed as ['residual' soil Fe_2O_3 (say, 2·0 mgm.) + error in concentrating and analysing[4] 4·0 mgm. Fe_2O_3]. Since the error of a normal iron determination has been found to be 6·5 per cent ($P = 0{\cdot}95$), the above error at the most is [2·0 + 0·26 ($P = 0{\cdot}95$)] mgm. = 2·26 mgm. Fe_2O_3, which reckoned on 32 mgm. is of the order of 7 per cent. This figure compares favourably with the normal analytical error, and small differences can be ignored when field-sampling errors are taken into account[5]. Further, from the nature of the calibration curves[1,6], we may write for values of $C_T \leqslant 1{,}000$ p.p.m. :

$$\log C_T = \log(C_t/C_S)_M + \log C_S + \log I_T/I_S$$

where C_t is the value of C_T when $\log I_T/I_S = 0$. If for $\log C_S$ we substitute $\log[100 \times 32/w]$ and subtract from it the log concentration factor = $\log[2 \times 10^4/w]$ (for 20-gm. aliquots of soil), we derive an expression for the C_T value in the soil, namely:

$$\log C_{T(\text{soil})} = \log\,(C_t/C_S)_M + \log \frac{32 \times 10^2/w}{2 \times 10^4/w} + \log I_T/I_S = \log 0{\cdot}16(C_t/C_S)_M + \log I_T/I_S$$

or

$$\log C_{T(\text{soil})} = \log k + \log I_T/I_S$$

where k is a constant determined by the calibration and experimental arrangement.

Regarding the proposed modification: (i) it neither interferes with the method of analysis nor does it introduce extra work, apart from the addition of Fe_2O_3; (ii) it renders unnecessary the analysis of internal standard content; since these analyses are usually performed in batches of six, each batch requiring about 2 hr. for complete assay, there is a substantial saving in operational time; (iii) it does not increase unduly the final weight of concentrate which is normally about 40–60 mgm.; and (iv) it avoids graphical calculation, thereby permitting concentrations for acetic acid-soluble cobalt to be computed directly from the photometric evaluation in the ranges 0·04–3·50 p.p.m. ('normal' 40 mgm. concentrate) and 0 03–3·00 p.p.m. ('normal' 60 mgm. concentrate). Since most extractable cobalt contents fall well within the above limits, the scheme may be applied with advantage in the routine analysis of available cobalt in soils: its general application would depend on (a) the amounts of acetic acid-soluble iron in the run of samples being analysed, and (b) the magnitude of w_1 and the subsequent dilution with Fe_2O_3 to within the limits imposed by the straight-line portion of the correction graph[1].

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ARCHÆOLOGY

An Extinct Giant Pangolin and Associated Mammals from Niah Cave, Sarawak

EXAMINATION of the mammalian remains excavated from the west mouth of Niah Cave, Sarawak[1], in 1954–58 down to a depth of 72 in., which corresponds to a carbon-14 date of about 32,000 years B.P. (ref. 2), established a fauna containing only the lowland evergreen rain forest species of modern south-east Asia[3]; of those identified, only the Malay tapir[4] and the tiger (an unerupted canine from Neolithic levels ($E/G1$, 6–12 in.)) are to-day unknown from the wild in Borneo.

Extension of the dig to lower levels has now produced three bones attributable to *Manis palaeojavanica* Dubois—an extinct giant pangolin formerly known only from Middle Pleistocene deposits in Java—from depths at Niah ($H/17$, 104–110 in.) corresponding to carbon-14 dates of about 42–47,000 years B.P. (ref. 5). Besides man (represented by skeletal remains as well as artefacts of stone and bone) the associated mammalian fauna includes only extant species. Represented are: pig (*Sus* cf. *barbatus*), orang utan (*Pongo pygmaeus*), monkeys (certainly *Presbytis* species, others indeterminate), a wild cat (*Felis* cf. *bengalensis*), a large bovine (*Bubalus* c.q. *Bibos* species), the larger mouse-deer (*Tragulus napu*), a number of rats (including *Rattus* cf. *sabanus*, cf. *mulleri* and cf. *rattus*), a shrew (*Crocidura* species), and several bats (identified by the Earl of Cranbrook) (including *Rousettus* cf. *amplexicaudatus*, *Hipposideros* cf. *diadema*[6], cf. *galeritus*[6], and two indeterminate species of intermediate size, *Chaerephon* species, *Cheiromeles* cf. *torquatus*[6] and *Myotiss* pecies[6]), as well as the common recent Malaysian pangolin (*Manis javanica*); the last has never before been found in the fossil or sub-fossil state. The Niah finds prove the co-existence of the two species of *Manis* in the Upper Pleistocene of Borneo.

Fuller reports will be published in forthcoming issues of the *Sarawak Museum Journal*.

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D. A. HOOIJER

Rijksmuseum van Natuurlijke Historie,
Leyden.

MEDWAY

Department of Anatomy,
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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, January 16

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. J. E. Thorpe and others: "A University Expedition to British Honduras".

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. R. Popper: "The Growth of Knowledge" (President's Address).

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. Hubert Martin: "Pesticides and the Public".

University of London (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. F. Bergel, F.R.S.: "Biochemistry and Biology of Enzymes in Cancer".*

Monday, January 16—Friday, January 20

Institute of Physics and the Physical Society (at the Royal Horticultural Society's Halls, Westminster, London, S.W.1)—45th Annual Exhibition.

Wednesday, January 18

British Institution of Radio Engineers, Computer Group (at University College, Gower Street, London, W.C.1), at 2–5 p.m. and 6–8 p.m.—Symposium on "Alpha Numeric Displays".

Institute of Physics and Physical Society, Acoustics Group (at the Royal Horticultural Society (New Hall), Vincent Square, London, S.W.1), at 2 p.m.—Symposium on "Traffic Noise". Speakers: Dr. D. W. Robinson, Mr. C. H. G. Mills and Miss S. W. Forgarty.

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. Oliver N. Millar: "The Restoration Portrait" (Fred Cook Memorial Lecture).

Institute of Physics and Physical Society, Low Temperature Group (at 47 Belgrave Square, London, S.W.1), at 4 p.m.—Annual General Meeting. 4.30 p.m.—Dr. R. W. Hill. "The Thermodynamic Properties of Fluid Helium".

Bone and Tooth Society (at the Institute of Orthopædics, 234 Great Portland Street, London, W.1), at 4.30 p.m.—Symposium on "The Temporo-Mandibular Joint".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Mr. J. S. A. Green: "A Problem in Baroclinic Stability"; Mr. F. Knighting: "Some Computations of the Variation of Vertical Velocity with Pressure on a Synoptic Scale".

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. M. N. John: "A General Method of Digital Network Analysis particularly suitable for use with Low-Speed Computers"; Dr. P. P. Gupta and Prof. M. W. Humphrey Davies: "Digital Computers in Power System Analysis".

Society of Chemical Industry, Corrosion Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. R. L. Samuel: "Metallic Diffusion Coatings".

Royal Institute of Chemistry (at King's College, Strand, London, W.C.2), at 6.30 p.m.—Prof. Sir C. N. Hinshelwood, O.M., F.R.S.: "Aspects of the Chemistry of Living Cells".

Wednesday, January 18—Thursday, January 19

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 2.30 p.m. on Wednesday and 10 a.m. on Thursday—Symposium on "Pressure Vessel Research Towards Better Design".

Thursday, January 19

Northampton College of Advanced Technology (at St. John Street, London, E.C.1), at 3 p.m.—Prof. Dimitri Riabouchinsky (l'Académie des Sciences de l'Institut de France): "Some Special Problems in Fluid Mechanics".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. E. C. Zeeman: "Geometric Topology".

University of London (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. A. N. Michelson: "Nucleotide Anhydrides and Oligonucleotides".* (Further lecture on January 26.)

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. R. P. Jordan: "Problems Associated with Research on Aggregates".

Pharmaceutical Society of Great Britain (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Mr. K. A. Lees: "Fermentation Processes in Preparing Pharmaceutical Materials".

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. T. Wilson: "Filariasis in Malaya".

Society for Visiting Scientists (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Atmospheric Pollution". Chairman: Dr. R. Lessing, O.B.E. Speakers: Dr. S. R. Craxford, Dr. P. J. Lawther and Dr. E. A. J. Mahler.

British Institute of Radiology (at 32 Welbeck Street, London, W.1), at 8 p.m.—Mr. P. E. Thompson Hancock, Mr. J. D. N. Nabarro and Mr. P. B. Kunkler: "Treatment of the Reticuloses by Chemotherapy and Radiotherapy".

Friday, January 20

Association of Applied Biologists (in the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.40 a.m.—Symposium on "Recent Developments in Plant Nematology".

Institute of Navigation (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Mr. A. Stratton and Mr. K. R. Honick: "Presentation of Height Information in Aircraft".

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Management Studies for Junior Engineers", opened by Mr. G. S. C. Lucas.

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. M. C. Whiting: "The Chemistry of some Macrocyclic Antibiotics".

Royal Aeronautical Society (in the Lecture Theatre, 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. Lorne Welch: "Gliding and Man-Powered Flight".

Saturday, January 21

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss R. Osborn: "High Life Among Gorillas".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Scientific Officer (with a good honours degree in chemistry or botany, preferably with some postgraduate experience) in the Weeds Section, to work on problems associated with the absorption, mobility and persistence of herbicides in soils—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (January 28).

Head of the Department of Science of the Jamaica College of Arts, Science and Technology, Kingston—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (January 31).

Lecturer (honours graduate in science) in the Department of Building Science—The Registrar, The University, Liverpool, quoting Ref. CV/N (January 31).

Lecturer in the History of Science—The Secretary, The University, 38 North Bailey, Durham (January 31).

Lecturer or Assistant Lecturer in the Department of Applied Mathematics—The Registrar, The University, Liverpool (January 31).

Research Assistant (with an honours degree in physics) in the Department of Mathematics, to continue and develop a fundamental investigation of the rheological properties of concentrated polymer solutions—The Registrar, The Manchester College of Science and Technology, Sackville Street, Manchester 1 (January 31).

Senior Lecturer (preferably active in magneto-fluid dynamics or plasma physics and able to initiate research in one of these fields) in Mathematics—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1 (January 31).

Lecturer (with a degree in science, preferably in chemistry) in the Department of Education—The Registrar (Room 22, O.R.B), The University, Reading (February 1).

Chair of Logic—The Secretary of University Court, The University, Glasgow (February 3).

Assistant, Assistant Experimental Officer grade (with a pass degree or equivalent qualification), in the Department of Potato Genetics, to help in biochemical investigations in potatoes—The Secretary, John Innes Institute, Bayfordbury, Hertford, Herts (February 7).

Lecturer or Assistant Lecturer in Pure Mathematics—The Registrar, The University, Leeds 2 (February 7).

Lecturer or Assistant Lecturer in Pure Mathematics; and a Lecturer or Assistant Lecturer in Applied Mathematics—The Secretary and Registrar, The University, Southampton (February 10).

Senior Lecturer (preferably with some experience in physical anthropology and comparative anatomy) in Anatomy at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 10).

Lecturer (with an honours degree in psychology and interests in experimental psychology) in Psychology at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 14).

Senior Technician (with training in histological techniques and the preparation of teaching material) in the Zoology Department, University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 15).

Senior Lecturer or Lecturer (with special qualifications, teaching and research experience in either biogeography and climatology, or cultural (especially economic) geography) in Geography at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 17).

Chair of Applied Mechanics tenable in the Newcastle Division of the University—The Registrar, University Office, 46 North Bailey, Durham (February 18).

Chair of Physics tenable in the Newcastle Division—The Registrar, University Office, 46 North Bailey, Durham (February 18)

JOHNSON AND JOHNSON RESEARCH FELLOW (with previous experience in tissue transplantation or related fields), to investigate problems of tissue transplantation—The Secretary, East Grinstead Research Trust, Queen Victoria Hospital, East Grinstead, Sussex (February 24).

CHAIR OF INORGANIC CHEMISTRY tenable in the NEWCASTLE DIVISION of the University—The Registrar, University Office, 46 North Bailey, Durham (February 25).

LECTURER or ASSISTANT LECTURER IN ELECTRONICS—The Secretary and Registrar, The University, Southampton (February 28).

DEMONSTRATOR IN INORGANIC CHEMISTRY—Dr. Lee's Professor of Chemistry, Physical Chemistry Laboratory, The University, South Parks Road, Oxford (March 1).

CHAIR OF METALLURGY within the Faculty of Engineering, University of Sydney, Australia—The Secretary Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 15).

JUNIOR MYCOLOGIST (with a good honours degree in botany, postgraduate training in systematic mycology, a working knowledge of Latin and German, with Russian an added qualification) at the Commonwealth Mycological Institute, Kew—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (March 31).

LECTURER IN RADIATION BIOLOGY—The Secretary, Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London, W.1 (April 1).

ASSISTANT, Grade B (with an honours degree in physics, research or industrial experience in some branch of applied physics, and preferably teaching experience) IN THE DEPARTMENT OF PHYSICS—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol 7.

ASSISTANT LECTURER (graduate in chemistry, and preferably with experience in the food industry)—The Principal, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.

ASSISTANT (young research worker interested in the electron microscopic structure of cells) IN THE CANCER RESEARCH UNIT—Prof. G. Causey, Department of Anatomy, Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2.

GEOLOGISTS (preferably under 35, a first- or good second-class honours degree in geology with emphasis on metamorphic and igneous rocks and/or structural geology, and preferably previous experience) in Tanganyika, for regional geological survey of metamorphic and igneous rock terrains, and preliminary investigation of mineral deposits—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.114/7/06.

GEOLOGIST (with a first- or upper second-class honours degree in geology) in the Bechuanaland Protectorate, to undertake general geological surveys, including the examination of mineral deposits, water supply problems and geological mapping—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/76/03.

OFFICER (with a degree in science, or diploma in horticulture or agriculture), to assist in field experimental work—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick.

PHYSICISTS (with a second-class honours degree or equivalent in mathematics or physics, and for the more senior posts, at least three years postgraduate research experience), to join a team engaged on an experimental programme on the Fast Reactor—The Personnel Manager, U.K.A.E.A., Dounreay Experimental Reactor Establishment, Thurso, Caithness, quoting Ref. 163/34.

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BROADCASTING AND TELEVISION IN EDUCATION

THE presidential address entitled "Science Broadcasting in Sound and Television" delivered by Mr. A. B. Oldfield Davies to the Assembly of Corresponding Societies at the Cardiff meeting of the British Association was certainly realistic, if not also somewhat pessimistic, as to the contribution of television or sound broadcasting to the effective diffusion of real scientific knowledge. Sound broadcasting, he thought, with its greater flexibility and variety of programme services, has some substantial advantages at present over television; but he seemed very doubtful whether even the most outstanding programmes do any more than help to create an atmosphere of respect for knowledge as something good in itself. That opinion is not lightly to be dismissed and is one to be kept in mind when the expansion of educational broadcasting is being so widely discussed. The British Broadcasting Corporation is, of course, placing on a permanent basis its school television service and doubling its output; it is also continuing its school broadcasting in sound, and also the Network Three service for programmes devoted specifically to the aims of further education. The development of transistor receivers, cheap and easy to run, has in recent years opened up immense possibilities for expanding the audiences for medium-wave transmissions in the less-developed areas, particularly in Asia and Africa, though in many parts of the world short-wave broadcasting is still the main means of communication.

These new possibilities were duly noted by the Ashby Commission on Post-School Certificate and Higher Education in Nigeria. Its report, "Investment in Education"*, contains a note on new educational techniques in which they are briefly discussed. Although it is recognized that teaching through television is in its infancy and much has yet to be learned in both the use and improvement of the medium, it is recommended that, while it is still problematic when Nigeria will be able to embark on television on a large scale, channels for educational television should be reserved now, in order to ensure that, when educational television becomes practicable, the opportunity will not have been lost because the channels have been appropriated for commercial television. This, of course, is a large part of the case for allotting a second channel to the British Broadcasting Corporation, as is emphasized in the Corporation's recent annual report† and in the important speech on the future of broadcasting in Britain which the director-general, Mr. H. C. Greene, delivered to the Manchester Luncheon Club on November 9 and which has since been substantially reproduced in *The Listener* (64, 875; 1960).

Other recommendations on the new educational techniques made by the Ashby Commission include experiments with the use of the record player and magnetic tape recordings in language teaching; the organization and maintenance of a central film library for distributing to schools on a loan basis; a determined effort to use radio for English broadcasts in primary schools for at least five grades; and establishment by the Federal Minister of Education of a committee to study advances in these fields and to make recommendations for experiments in Nigerian schools. These recommendations were in fact duly noted by Lord Newton in replying for the Government on an important debate on modern aids in education in the House of Lords on November 29; and it is obviously desirable that the significance and potentialities of all such developments should receive full consideration by the Pilkington Committee on the Future of Sound and Television Broadcasting, which already has before it conflicting views as to the allocation of a third television channel. Meanwhile, it should be noted that the Minister of Education, Sir David Eccles, in opening the annual conference of the National Institute of Adult Education at Swansea, suggested the screening of television programmes of adult education. The Organization for European Economic Co-operation has already advocated the use of school television broadcasts as a contribution towards overcoming difficulties arising out of the shortage of science teachers, and, although the debate in the House of Lords brought out some misgivings on the part of school teachers, it is clear that there is wide opportunity for experiment and indeed research into the use of both sound and television broadcasting for educational purposes. Indeed, a report published last May by the University of Leeds advocated entrusting any third television channel to a Universities Television Corporation rather than to the British Broadcasting Corporation or to the Independent Television Authority.

Such a network, it was suggested, should be dedicated to education and the dissemination of knowledge, speculation and the arts, and the suggestion arose out of an examination of teaching methods. It was intended to make lectures by leading specialists available to large numbers of students at the same time, and the proposal was also concerned with the maintenance of the closest possible link between a university and its town or region. Calling for the exploitation, not the resistance, of television, it visualized spreading far more widely the activities and influence of the extra-mural departments of the university. Also the network would have at its disposal the best academic minds of the country. It would be able to provide a series of courses directed at different levels of intelligence, but possessing more depth than those now attempted; and the proposal also contemplates facilitating contact of the universities with secondary school children through its television programmes instead of merely through examinations.

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* Investment in Education: The Report of the Commission on Post-School Certificate and Higher Education in Nigeria. Pp. v+140. (Lagos: Federal Government Printer; London: Crown Agents for Oversea Governments and Administrations, 1960.) 5s. net.

† Annual Report and Accounts of the British Broadcasting Corporation, 1959–60. Pp. 175+8 plates. (Cmnd. 1174.) (London: H.M. Stationery Office, 1960.) 9s. net.

This project was briefly noticed in the debate in the House of Lords, but not in Mr. Greene's speech in Manchester on November 9. Apart from a strong plea for public service broadcasting in the sense in which it had been defined by Sir Ian Jacob—as a compound of a system of control, an attitude of mind and an aim which, if successfully achieved, resulted in a service which could not be given by any other means—Mr. Greene was concerned more especially with local broadcasting and the possibility of serving minority interests, which, in a particular and limited sense, a university television network might also be said to do. He quoted Sir Ian Jacob's conception of the system of control, as full independence, or the maximum degree of independence that Parliament would accord; of the attitude of mind as an intelligent one capable of attracting to the service the highest quality of character and intellect; and the aim to give the best and most comprehensive service of broadcasting to the public that is possible. He then stressed the vital factor of the underlying motive, which must not be vitiated by political and commercial considerations. The support which was given to Lord James of Rusholme's remark in the House of Lords debate in reference to the peak viewing hours, that we have sold our broadcasting system to advertising, indicates how much agreement there is here with Mr. Greene's argument.

Mr. Greene sought to show how, precisely because of this situation with regard to the peak hours, only a public service system could ensure for the local audience a genuinely local and independent programme. In support of the British Broadcasting Corporation's claim, he adduced its experience in resisting political and commercial pressures, which could be intense at the local level, in collaborating with universities, technical colleges and schools in providing educational programmes, and in the impartial reflexion of all kinds of controversies. He also mentioned the support of the Corporation's nationally based service in collecting items for the local audience and the existence of its three national networks as sources of programmes at times when the local station is not putting out its own programmes. He is convinced that there is plenty of good material for local broadcasting and that such broadcasting has an essential contribution to make to the life of the community, though he maintained that this can only be made within the framework of public service broadcasting, and doubted whether a universities television network could prove effective enough to resist pressure or be efficient enough in making use of the channels available.

Mr. Greene was concerned rather with low-powered transmitters operating over a radius of no more than four or five miles; in such a network of local stations, sound radio could well provide a service beyond the reach of television and tap the neglected wealth of resources in the universities and elsewhere as well as assist in promoting a more vigorous local culture. Mr. Greene has been criticized for his omission of any reference to religious broadcasting in this connexion, and there is clearly something to be said for any local VHF stations including local councils of churches with other local institutions with which they can work in harmony while maintaining standards of broadcasting. Apart from this, the fact that a Cambridge firm of television and radio equipment manufacturers has already outlined a scheme for establishing a hundred local VHF radio stations in Britain, run, not as part of a big organization, but by individual owners as local affairs, and with the transmitter available to the education authorities for schools broadcasting during the day when it is not in use for the main transmission, confirms that local sound broadcasting is already practicable. Apart from the scheme outlined in this pamphlet issued by Pye, Ltd., about a hundred companies have in fact been registered with the object of running such local stations if and when it becomes possible.

In opening the debate in the House of Lords, Lord Bessborough suggested that the very success of sound radio in education is one potent reason why no more than 2,500 out of some 30,000 schools in Britain take the educational television programmes of the British Broadcasting Corporation and of Associated Rediffusion. He agreed with Mr. R. Hickman, of the National Union of Teachers, that many more programmes are needed, but that it is essential to ensure the utmost co-ordination and co-operation among initiating companies, who must build up and maintain units able both to become expert in teaching methods and techniques, and also to maintain the closest possible contact with schools. Lord Bessborough urged also that full advantage should be taken of special opportunities in universities, and particularly that universities should be enabled to start pilot schemes; and although Lord Newton took up this point in his reply, he suggested that the initiative should come mainly from the universities themselves, and that the Pilkington Committee might express opinions on the merits of university broadcasting and television.

Much the weightiest speech in the debate was that of Lord James of Rusholme. He acknowledged the very great contribution which television—and still more sound broadcasting—has made to education in Britain, and paid tribute to the work of the British Broadcasting Corporation, particularly in research and development, and to that of the Schools Broadcasting Council, through which the Corporation has worked. There is still need, Lord James thought, for much research in the use of broadcasting, and we might be on the threshold of fundamental developments. There are also the limitations imposed by finance, and he strongly supported not only the plea put forward by the Corporation in its current report for the retention of the whole of the licence fee but also increasing the fee to an economic figure. The effective use of television, however, depends ultimately on the imagination and skill of the class-room teacher, and he thought it futile to over-emphasize the value of television in the schools when these programmes have to contend with the 'diseducation' effect of those broadcast at the peak hours for child-viewers—between 6 and 9 p.m.

Other speakers in the debate, like Lord Aberdare, Lord Strang and the Baroness Swanborough, stressed the importance of research and of the influence of the teachers, which, it was emphasized, are not replaced either by sound or by television programmes, and Lord Newton readily responded. There is no question, he said, of attempting to meet the present shortage of teachers by trying to use broadcasts as a substitute for teachers, and he suggested that the main function of broadcasting in Britain, both on sound and on television, is enrichment of teaching, as opposed to the direct teaching method of the kind used in the United States. As Lord Newton understood it, this meant that broadcasts could reinforce a teacher, extend the range of teaching and provide fresh help in the classroom, and he thought that among teachers there is much serious thought and goodwill and the recognition that this new medium is something to be taken seriously and of great potential importance. Speaking about equipment, he merely observed that the Minister of Education has no direct financial control over the purchase of equipment for the purpose; this is entirely a matter for the local education authorities. He agreed as to the need for more research, but referred to the work of the National Committee for Visual Aids in Education, and stated that the report of the Organization for European Economic Co-operation, "Teaching through Television", is being studied by the Minister of Education. It is the policy of the Colonial Office to encourage development of sound and television broadcasting on local lines and with the use of local talent. Sympathetic consideration is also being given to financial aid for a further period to the Overseas Visual Aids Centre in Tavistock Square.

Lord Newton's hope that this debate would stimulate constructive thought in educational circles about the possibilities of school broadcasts and television should certainly be fulfilled, and his view that the great trusts and foundations could give a most useful lead in research in this field should be duly noted. Lord Newton thought, too, that the teachers' attitude would be a main determining factor, and that this might be particularly influenced by the results of research. As yet, however, as was almost universally acknowledged in the debate, sound broadcasting is by far the more important, and a prime task accordingly is to see that the resources and techniques available are adequate and appropriate. Nevertheless, although the British Broadcasting Corporation's views on local broadcasting, as set forth both by the Director-General himself and in the Corporation's current annual report, embrace full and free association and co-operation with universities, local authorities, newspapers and other important institutions within the local communities, there is an influential volume of opinion, not reflected in the debate in the House of Lords, which is strongly opposed to any extension of the British Broadcasting Corporation's power in this field. That the British Broadcasting Corporation is seriously misrepresented in this connexion may be of relatively little importance: some of the misrepresentations have been decisively refuted by Mr. Greene. It is more serious, however, that the picture of local broadcasting in other countries should also be distorted in this same connexion, and more debates, free from party political bias and commercial misrepresentation, are undoubtedly needed if a sound judgment is to be reached as to the manner in which educational broadcasting, whether in sound or television, should be approached. Further consideration is required of the way in which the universities and schools might co-operate, and how in the development of local broadcasting and television the conception of public service could best be preserved and to ensure that there is no sacrifice of the public interest—educational or cultural—to sectional or purely financial advantage.

THE CORRESPONDENCE OF ISAAC NEWTON

The Correspondence of Isaac Newton, Vol. 2
1676–1687. Edited by Prof. H. W. Turnbull. Pp. xiii + 552 + 6 plates. (Cambridge: At the University Press, 1960. Published for the Royal Society.) 147*s*. net.

THE decade (1676–87) spanned by the present volume was an eventful one in Newton's life. While he was harassed by a rather nasty controversy with the Liège Jesuits about the theory of colours, he had to face, in friendly rivalry, Leibniz's first claim to the independent discovery of the infinitesimal calculus (1676–78). Then (1679–80) came the fateful discussion with Hooke about the fall of a body to the centre of the Earth, which led Newton's thoughts to the more general problem of finding the trajectory of a gravitating body. He soon received a further incentive to pursue this line of investigation from Flamsteed's controversial interpretation of comet observations at the end of the year 1680 (Old Style). Finally, prompted by Halley's famous visit in 1684, he was induced to write down his decisive results and to elaborate them during the ensuing years (1684–87) in the form of the *Principia*.

This succession of dramatic incidents fills the pages of this fascinating volume, in which are assembled not only the well-known documents which form the basis of existing biographies but also a large number of hitherto unpublished letters and manuscripts which throw much new light on the events and on Newton's complex personality. The very excellence of the editor's work elicits in but one respect an expression of regret: one would wish that he had been somewhat more generous in the inclusion of auxiliary documents not strictly of epistolary character; for example, from the important accounts of the work done in Great Britain on infinite series, drawn up by Collins for the information of Leibniz, we are only given those passages which refer directly to Newton; this documentation, however, is too fragmentary to be of any use to the serious student, and one may question very much the wisdom of such an effort at economy. It is true that these documents, as well as others equally essential, like the treatise *De Motu*, exist in print; but the books in which they are published have practically vanished from the market and are even not common in public libraries; a reprint in the context of the *Correspondence*, which would not have sensibly increased the bulk of the volume, would therefore have been most welcome. Perhaps an earnest plea for a somewhat broader conception of the selection of material may still have some influence on the preparation of future volumes.

One hitherto quite unknown episode, revealed by the present publication, is the prolongation of the futile controversy about Newton's theory of colours started by the English Jesuits of Liège: one assumed that it was closed by the last answer from Newton published in the *Philosophical Transactions* in 1676 (Letter 173 in this volume), but we now learn that the exchange of letters between the Jesuit Lucas and Newton went on at intervals during two more years, the former becoming more and more venomous and the latter increasingly rude. Obviously, the mixture of cunning casuistics and pedantic irrelevancy displayed by his opponent was too much for Newton's robust common sense and sound logic, but he had also a deeper motive for so insistently pursuing a pointless and sordid quarrel: Lucas had found for the elongation of the solar spectrum a smaller value than Newton, and this sufficed to rouse the morbid suspiciousness which the latter manifested whenever he thought his accuracy and veracity impugned. Lucas's attempts at belittling the discrepancy only increased Newton's exasperation, and unfortunately made him blind to the true explanation—the difference in dispersive power of the glasses, although this was actually suggested (Letter 200) (amid a number of irrelevances) by Lucas himself! Hooke (Letter 223) wondered at Newton's "patience"—unaware of this tragi-comic aspect of the situation.

At the same time, through the intermediary of Oldenburg, a thorough confrontation was taking place of the respective achievements of Newton and Leibniz and their respective followers in the treatment of various algebraical and geometrical problems by a method of series expansion, involving a limiting process of derivation or differentiation as well as the inverse process of integration. To this correspondence Newton contributed, somewhat reluctantly, two remarkable Letters (165 and 188), of which especially the second ("epistola posterior") is a document of capital interest, retracing as it does in great detail the successive steps through which Newton arrived at the full realization of his method. The whole material pertaining to this momentous development (unfortunately with some curtailment, as mentioned above) is here fully elucidated by the editor's especially careful and elaborate annotation.

We then pass to the no less remarkable circumstances which led to the genesis of the *Principia*. The first act is well known already by the accounts given by Rigaud and Rouse Ball, and, quite recently, by J. Lohne in *Centaurus* (vol. 7, pp. 6–52; 1960). Challenged by Hooke's acute criticism, Newton is forced to rectify his views concerning the path by which a falling body would eventually reach the centre of the Earth; and from this academic problem he is further led (as we know from the later correspondence with Halley) to study the dynamical behaviour of bodies acted on by gravitation. It appears that the crux of this problem was neither the formulation of the inverse square law (which had been inferred from Kepler's laws by Hooke, Halley and Wren as well as by Newton), nor the conception (inspired by Galilei's treatment of projectiles) of the planetary motion as a uniform rectilinear one modified by an attraction from the Sun; the difficulty was to determine the shape of the resulting trajectory. This was the decisive step which Newton then (1680) took; but, as usual, after solving the problem, he "threw the calculation by, being upon other studies".

It was not long, however, before his attention was again turned to the same question, this time by a concrete case of astronomical observation. In the last months of the year 1680 (Old Style) two comets had been successively observed, the first moving towards the Sun, the second away from it. Flamsteed, who had himself contributed to these observations, suggested that the two objects were the same comet, first attracted, then repelled by some magnetic action of the Sun; and he solicited Newton's opinion. The ensuing correspondence is now for the first time published in full, and it enables us to form a picture of this incident rather different from the somewhat misleading account of it given by Brewster. Flamsteed's magnetic theory, as one imagines, did not resist Newton's remarkably sound criticism, based on the empirical knowledge of magnetic phenomena; but at the same time, Newton accurately described the orbit which a single comet could have followed under the action of gravity: at first, he hesitated to accept the proposed identification of the two objects on the ground that there was no precedent for such a form of cometary orbit, all the known ones being practically rectilinear. However, as better observations became available, it became clear that they did fit a single orbit of the type indicated by Newton.

It is not surprising, therefore, that when Halley, in August 1684, asked him what would be the trajectory of a gravitating body under the inverse square law, Newton had the answer ready. (Lohne's surmise, expressed in the aforementioned article, that Newton did not then have the proof, but only worked it out after Halley's visit, is not in harmony with Newton's own declaration, nor with other circumstantial evidence.) The story of how Halley succeeded in persuading Newton to write the *Principia*, how Hooke's not unjustified reclamation of priority nearly wrecked the enterprise, how Halley's tact and devotion rescued it and carried it to a good ending, is too well known to need repetition. But it is a delight to read it again, in the protagonists' own words, so faithfully recorded and beautifully printed.

L. ROSENFELD

ATOMS AND MOLECULES

Basic Physics of Atoms and Molecules

By Dr. U. Fano and L. Fano. Pp. xv+414. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 80*s*. net.

THE book is divided into two parts and ten appendixes. The first part presents the basic facts concerning the structure of matter and introduces the new concepts required to correlate them. The second part deals with aggregates of particles, being chiefly concerned with chemical bonding. Mathematical background is provided in the appendixes.

At an early stage most text-books introduce experimental evidence concerning the wave aspects of particle beams, and, from a comparison with beams of light, the Schroedinger wave-equation quickly follows. This book arouses interest because the authors prefer to discuss the analogy between polarization of light beams and of molecular beams. They consider that discussion of a system having a small number of eigen-states is more instructive, and, in addition, polarization in the classical treatment of radiation has much in common with ideas of quantum mechanics. The analogy brings out clearly the principle of superposition, but their picture is somewhat clouded by a premature introduction of rotational matrices, the mathematics of which has fortunately been relegated to an appendix.

The second part will appeal more to the chemist than the physicist, who would prefer an account of metals and semi-conductors and a more detailed description of the simple molecules. Indeed, the chemist may find the whole of this part too brief and qualitative.

Finally, it is difficult to decide for whom the book is intended. The authors state in their preface that it is directed primarily to research workers in the natural sciences (and undergraduates) and presupposes only a general knowledge of physics and mathematics. This view is supported when, in two footnotes, they find it necessary to explain what is meant by the exponential function, and in the first few appendixes when among other subjects they describe the relative motion of two particles and introduce the concept of complex numbers. The next appendix gives the geometrical calculation of rotational matrices and is followed by an equally difficult one on the mathematical formulation of quantum laws. A mathematical derivation of the Uncertainty Principle is given, whereas the qualitative treatment involving the 'gamma-ray microscope' may have been more consistent with the aims of the preface.

On the whole, the average student will find this book particularly heavy going. G. A. Jones

RACIAL PREJUDICE AND MENTAL HEALTH

Race Relations and Mental Health

By Marie Jahoda. Pp. 48. (Paris: Unesco; London: H. M. Stationery Office, 1960.) 1.75 NF.; 2*s*. 6*d*.; 0.50 dollars.

THE virulence with which racial prejudice has made its appearance in the present century is one of the strangest and most disturbing phenomena of present times. Race hatred and conflict thrive on scientifically false ideas and are encouraged by ignorance. To illuminate errors in fact and reasoning and to make known conclusions reached in various branches of science and to combat racial propaganda, the Director-General of Unesco was asked to study and collect scientific material concerning questions of race; to give wide diffusion to the scientific information collected; and to prepare an educational campaign based on this information.

Before this programme could be carried out, it was necessary for Unesco to have the relevant facts at its disposal. Accordingly, a number of prominent scientists were asked to summarize the main results in the field of their specialization. Some of them have already presented the results of their investigations in booklet form, and, now, Marie Jahoda examines in *Race Relations and Mental Health* the psychogenetic bases of prejudice and the relationship between prejudice and mental health. The work is commendable for the objective way in which it is presented and, where the author presents her own views, makes it clear that they represent her opinions.

Whether racial antagonism is based on fact or fancy becomes a question of the adequacy of inference; there is a growing body of empirical evidence to show that inadequate reality-testing is characteristic of many who feel hostile to racial outgroups This is well brought out by Jahoda who shows that the same qualities which are given as reasons for disliking another racial group are often highly appreciated when found in a member of a favoured group. Another cause of prejudice is that the human propensity to make generalizations—and prejudgments—often results in some economy of mental effort. Such pre-judgments can harden into rigidly stereotyped thinking which eschews reality-testing even when facts are available.

After a psycho-analytical study of the deeper causes of prejudice, Jahoda discusses whether or not prejudice is inevitable. Here she refuses to be spuriously optimistic. Racial antagonism, according to her psycho-analytically oriented interpretation, is a deeply meaningful support to the individual of low mental health. The psychological need which leads to racial antagonism is universal and will continue to exist during the foreseeable future. Few will disagree with this conclusion or with her personal belief that "to deprive one's sense of identity from work or from stamp collecting or from mountain climbing may be psychologically as precarious as to deprive it from prejudice against underprivileged groups; but it may help one to live without hating either oneself or one's neighbours".

T. H. Hawkins

CHRISTIANITY AND SCIENTIFIC PROGRESS

Science, Technology and the Christian

By Prof. C. A. Coulson. (The Beckly Social Service Lecture, 1960.) Pp. 111. (London: The Epworth Press, 1960.) 8*s*. 6*d*. net.

PROF. COULSON'S Beckly Social Service Lecture is addressed primarily to those who share his Christian faith, for, in accordance with the purpose for which the Lecture was instituted, he seeks to show why the Christian, as such, should be concerned with technology as well as with science, the nature of the issues that are involved, and the manner in which Christians could contribute to the solution of some of the problems that arise, for example, in the control of nuclear power, the application of automation, or in food and population policies. With Archbishop William Temple, he regards Christianity as the most materialistic of religions, and in the same sense. Much in this Lecture accordingly should be welcomed by the scientist or technologist who shares that sense of the ethical values which Smuts and others since, like Hinshelwood, Appleton and Ashby, have expounded, whether or not Prof. Coulson's own faith is fully accepted. This attempt to stir Christian people to constructive thinking and the facing of change leads, for example, to an eloquent plea for an educational system which allows no child to pass through it without some acquaintance with both science and technology.

In a way, the Lecture could be described as an attempt to interpret in a specific Christian context the essential message of the Duke of Edinburgh's Study Conference at Oxford in 1956. The challenge of change is presented just as clearly, and Prof. Coulson brings out the social and human issues involved, to the solution of which he is convinced that Christian principles of conduct have an essential contribution to make. Here, however, the emphasis on the pace of change and the decay of tradition recalls a fine passage in A. N. Whitehead's *Adventures of Ideas*, which discusses routine, tradition and change and their implications in the modern world.

Prof. Coulson is never trivial and writes with a sincerity, dignity and restraint that should enlist the support even of those inclined to take umbrage at his claim that some of his principles are specifically Christian, in the immense task to which he calls us of seeking to use creatively and imaginatively all the vast powers now at the disposal of mankind, with the sensitive insight into human needs, the profound respect for human personality and the confidence that the world has learned particularly from the life of Christ. Prof. Coulson's scholarship and wide reading characterize this short book as profoundly as his human sympathy and his Christian faith, and although it is unfair to expect too much in such matters of a published lecture, regret may be entertained that the book loses something of its full value as a stimulus to further study through the absence of an index and all but a handful of references.

R. BRIGHTMAN

FROM ARTS AND CRAFTS TO TECHNOLOGY

A Short History of Technology from the Earliest Times to A.D. 1900

By T. K. Derry and Trevor I. Williams. Pp. xviii + 782. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 38s. net.

AS the large five-volume *History of Technology* was being written by Prof. Charles Singer and his colleagues, an abridged edition was planned to meet both the needs of a wider readership (the larger handbook unfortunately being beyond the means of the average reader) and the desirability of establishing more firmly in the minds of both historians and engineers the relation between the history of technology and history in general. Imperial Chemical Industries have again generously supported this venture. The fortunate fact that one of the authors, Dr. Trevor I. Williams, is not only well known as the editor of *Endeavour* but was also for several years engaged in assisting Prof. Singer in producing his pioneer handbook has made it possible to use the evidence and manuscripts collected in compiling the five-volume work. This is also the source of most of the 353 figures used in the text of this new book.

However, the authors have by no means produced merely an abridgment of Singer's handbook. They have planned their own "technological history and history of technology", in which the essential trends and developments are presented in a concise and very readable form. A good bibliography, designed for the English reader, points the way for further detailed work on the subjects broached in the various chapters.

The book consists of two sections, each of which is preceded by a "General Historical Survey" of the period covered. The first section "From the Earliest Times to A.D. 1750" takes up about one-third of this 700-page text-book. In nine thirty-page chapters we are introduced to the crafts necessary for producing food and satisfying other domestic needs, mining and metallurgy, transport, recording and communications, sources of power and the earliest forms of the chemical industry, and their history is carried up to the Industrial Revolution.

The remaining two-thirds of the book and more than 220 illustrations cover "The Industrial Revolution to A.D. 1900". This upper limit has been quite rightly chosen by the authors, as "by 1900 the most advanced societies were beginning to register a change in the aims, as distinct from the methods, of technology. For man was no longer pitting his wits against Nature merely for the easier satisfaction of long-felt wants. . . . With the entry upon a new period, in which necessity ceases to be the mother of invention", the inventor began to create new wants, and technology is embedded firmer than ever in the general history of civilization.

The last section deals with many tools, methods and machines, with which most of us are still familiar, even if some forms of technology are now slowly disappearing. Here we are confronted with the history of the steam engine and turbines, machine tools and the development of interchangeable machine parts. New materials such as wrought and cast iron, steel and concrete begin to play a prominent part. Transport is transformed by the coming of the steamer, railways, bicycles, motor-cars and aeroplanes. Coal-mining grows and fosters the cheap production of iron and steel and initiates the coal-gas and coal-tar industries. Pure synthetic chemicals such as soda, sulphuric acid, nitric acid and ammonia are basic products for a new chemical industry. Rubber and petroleum create the 'motor age', and there is the new electrical industry which provides better communications, lighting and power. Finally, there is modern mechanized farming and the processing and preservation of farm-products by canning and refrigeration.

These and many more subjects are here presented in a form which will prove to be most valuable to the historian without technical training, who, in his turn, will be confronted with the gaps in our knowledge for which he will be called on to provide the clues for further research, together with the engineer and the technologist. Seven tables relating political and economic historical data to those of technology are given at the end of this book, which is without doubt a most valuable introductory manual to a historical discipline still in its pioneering age.

R. J. FORBES

Algebraic Curves

By Prof. J. G. Semple and G. T. Kneebone. Pp. xiii + 361. (Oxford: Clarendon Press; London: Oxford University Press, 1959.) 45*s*. net.

RECENT work on algebraic geometry has been much concerned with placing the subject on foundations which should be genuinely algebraic; a programme set on foot by van der Waerden some twenty-five years ago. The theory of algebraic curves is a very important section of algebraic geometry, and while R. J. Walker's excellent treatise, based on methods of modern algebra, has been available for ten years, an account on similar lines, but tailored to suit the student in his final undergraduate or first postgraduate year, has been needed. This need has now been fully met. Semple and Kneebone start from the beginnings, and take the general theory as far as the Riemann–Roch theorem; the exposition is always clear and precise, and each chapter begins with a survey of what is to be found therein and concludes with valuable exercises and notes. No very extensive algebraic equipment is required in the early stages, and to make the work self-contained an appendix summarizes practically all the needed work on rings, fields, polynomials and elimination theory. Two chapters initiate special studies: that on "Coordinates of a Curve" is a most helpful introduction to the Cayley form for algebraic varieties, while that on "Local Geometry in S_2" deals with a logical algebraic basis for what used to be called 'indefinitely near points'. It should be added that references to the older methods involving algebraic functions and Riemann surfaces are not absolutely excluded. An older generation might ask what Salmon would have made of all this, but, remembering that according to his contemporaries Salmon was "never a calculus man", perhaps he would have come to appreciate this new close convolution of geometry with algebra.

T. A. A. Broadbent

Chemical Analysis of Resin-based Coating Materials

Edited by C. P. A. Kappelmeier. Pp. xxvii + 630. (New York: Interscience Publishers Inc.; London: Interscience Publishers, Ltd., 1959.) 147*s*.

THIS collection of papers on the analysis of coating compositions was edited by an expert in this particular field, the late Dr. C. P. A. Kappelmeier, who studied under Baeyer and Wieland.

The book contains practical contributions from twenty-six senior chemists of many leading chemical firms situated in all parts of the world. It constitutes a unique and welcome compilation of tested classical and modern methods for the analysis of resins, plastics, paints, solvents, etc.

C. Schwarzenback and W. Schneider present an excellent chapter on the complexometric analyses of metallic additives: A. Z. Conner describes the techniques of paper and columnar chromatography, but gas-liquid chromatography receives only brief mention. Infra-red and ultra-violet absorption methods receive detailed treatment, and even the applications of mass spectrometry are succinctly discussed in the 'rosin' section.

Specialized chapters are devoted to alkyd and synthetic thermoplastic resins, latex paints, cellulose lacquers, drying oils, etc., and much helpful laboratory detail is provided. This volume will undoubtedly prove a most valuable reference work for industrial analysts who are concerned with the quantitative and qualitative examination of the wide range of coating compositions now being manufactured by the chemical industries. It is, unfortunately, rather highly priced for a book of 630 pages, but it is most legible and printed on excellent paper.

D. T. Lewis

Microbiology

Historical Contributions from 1776 to 1908 by Spallanzani, Schwann, Pasteur, Cohn, Tyndall, Koch, Lister, Schloesing, Burrill, Ehrlich, Winogradsky, Warington, Beijerinck, Smith, Orla-Jensen. Edited by Raymond N. Doetsch. Pp. xii + 233. (New Brunswick, N.J.: Rutgers University Press, 1960.) 5 dollars.

THIS book follows a commendable trend in American scientific publishing during recent years of presenting, in abridged form, excerpts from the works of the great scientists of the past. For those of us—the majority—who will never have an opportunity of reading many of the original papers, such syntheses provide an admirable substitute. As the editor wisely points out, the selection of papers is necessarily arbitrary, and there is bound to be difference of opinion regarding inclusions and omissions. Nevertheless, few will quarrel with the majority of the works included here, each of which is prefaced with a brief biographical introduction and a picture of the author.

The collection will be of value to anyone interested in the history of science, and would be a useful adjunct to any course covering this period of scientific history. It should prove particularly valuable in schools where the historical aspects of scientific discovery tend to be overlooked. The diversity of subjects covered provides a fascinating insight into the manifold applications of the so-called scientific method.

W. H. Dowdeswell

Transactions of the International Astronomical Union

Vol. 10: Tenth General Assembly held at Moscow, 12–20 August, 1958. Edited by D. H. Sadler. (International Council of Scientific Unions: International Astronomical Union.) Pp. ix + 774. (Cambridge: At the University Press, 1960.) 90*s*. net.

THIS volume contains the Proceedings of the meeting of the International Astronomical Union held in Moscow (1958). It contains the resolutions adopted at that meeting and the reports and proceedings of the various Commissions within the Union. The reports of the Commissions provide valuable summaries of the state of the subject and provide extensive bibliographies of original research done, in the preceding three years, in those fields which come within the scope of the Commissions. Four joint discussions (solar flares and corpuscular streams, nucleogenesis in stars, the luminosity of cepheids and astronomical observations with artificial satellites, rockets and balloons) which took place during the meeting are reported. A list of members of the Union and their addresses, correct to October 1959, together with the membership of the Commissions, is also appended.

It is noted that the General Secretary is contemplating action to expedite the publication of the *Transactions*. The rapid publication of the *Transactions* in smaller volumes would certainly enhance their usefulness as a reference work.

D. McNally

EFFECT OF ALPHA- AND BETA-MELANOCYTE STIMULATING HORMONES ON THE SKIN COLOUR OF MAN

By PROF. AARON B. LERNER and DR. JOSEPH S. McGUIRE

Section of Dermatology, Department of Medicine, Yale University School of Medicine, New Haven, Conn.

SIX years ago it was shown that intramuscular injection into man of a relatively crude alkali-treated extract from hog pituitary glands produced darkening of skin and nevi[1]. This darkening was similar to that observed in patients with adrenocortical insufficiency and some types of pituitary tumour. The pituitary fraction used at that time had a moderate amount of melanocyte stimulating hormone activity, but almost no other measurable hormonal effect. Later, two types of melanocyte stimulating hormones, α- and β-, were isolated from the pituitary glands of hogs, cows, sheep, horses, monkeys and human beings[2-4]. The structure of α-melanocyte stimulating hormone in all species is the same, although it has not been completely established for man. On carboxymethyl-cellulose columns, α-melanocyte stimulating hormone from man behaves the same as that from other species[5]. On the other hand, there are at least five varieties of β-melanocyte stimulating hormone; and it appears that in some species two forms may exist (Fig. 1)[7]. Since the pituitary material used in the original experiments in man was crude and because alkali treatment prolonged the melanocyte stimulating hormone activity as tested with frog skin *in vitro*, it was desirable to repeat the experiments with pure α- and β-melanocyte stimulating hormones. In addition it was possible to refine the techniques for measuring changes in skin colour.

Traces of vasopressin in our best preparation of α-melanocyte stimulating hormone prevented us from using this material in human beings. Fortunately, we were able to use synthetic α-melanocyte stimulating hormone generously provided by Dr. Klaus Hofmann and his colleagues, who accomplished its synthesis in 1959[8]. Although the synthetic α-melanocyte stimulating hormone, unlike the naturally occurring hormone, had a formyl group on the epsilon amino-group of lysine, and glutamine in place of glutamic acid, its activity on frog skin was the same as that of the natural hormone. 100 mgm. of β-melanocyte stimulating hormone was prepared in our laboratories from hog pituitary glands by Dr. Teh Lee and Mrs. Vina Buettner-Janusch, and another 100 mgm. was supplied by Drs. R. Guillemin and A. V. Schally.

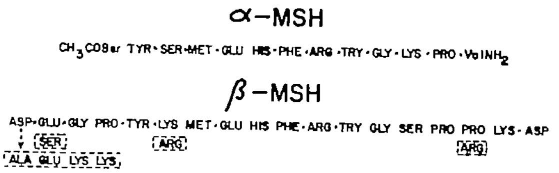

α–MSH
CH₃COSer TYR·SER·MET·GLU HIS·PHE·ARG·TRY·GLY·LYS·PRO·ValNH₂
β–MSH
ASP·GLU·GLY PRO·TYR·LYS MET·GLU HIS PHE·ARG·TRY GLY SER PRO PRO LYS·ASP
SER
ARG
ARG
ALA GLU LYS LYS

Fig. 1. Structure of α- and β-melanocyte stimulating hormones showing the common sequence of amino-acids in both peptides. α-Melanocyte stimulating hormone from hogs, cows, sheep, horses and monkeys is the same. The sequence of hog β-melanocyte stimulating hormone is given, and the sites of variation in different species are shown. Thus in cows and sheep serine replaces glutamic acid; in monkeys arginine replaces lysine (refs. 5, 6); in horses arginine replaces proline (ref. 6); and in human beings the tetrapeptide Ala·Glu Lys Lys is added to the N-terminal aspartic acid, and arginine replaces lysine (ref. 4). In some species, for example, sheep and monkeys, both glutamyl and seryl β-melanocyte stimulating hormone are present (ref. 7)

Four Negroes took part in the work. From the early experiments we found that, within limits, the darker the individual the greater his response to melanocyte stimulating hormone. Short supplies of both α- and β-melanocyte stimulating hormones necessitated short-term experiments with darkly pigmented subjects. Throughout the experiment changes in colour of the skin were recorded with two kinds of reflectance measurements and two kinds of colour photography. Photographs before and after each experiment were taken on the same roll of film so that the problem of variability in processing the colour film would not occur. Professional 'Ektachrome *E*-3' was used. Illumination was provided by three electronic flash units, and an Eastman 81*A* colour correction filter was used. All exposures were f.8. Also, 16-mm. 'movies' with colour film were taken of the face for 2–5 sec. daily. Reflectance measurements of the centre of the forehead and of the back of the forearm were made with a Cary model 14 recording reflectance spectrophotometer using light of 3500–7000 Å. Measurements of the forehead, cheek and arm also were made with a Photovolt model 610 photoelectric reflexion meter without filters and with red, blue and green filters as described earlier[1]. The injections, each 1·0 ml. in volume, were administered intramuscularly into the deltoid muscle. The melanocyte stimulating hormone was first dissolved in saline and then diluted with an equal volume of 16 per cent gelatin solution. The final concentration of melanocyte stimulating hormone usually was 4·0 mgm. per ml. This preparation was used in all subjects except J. R., who received the hormone in saline alone. Three of the subjects were employed at indoor jobs during the day. The fourth (R. R.) was unemployed for the duration of the experiment. All subjects avoided exposure to sunlight so far as it was practical and wore caps and shirts with long sleeves whenever they were out of doors.

Subject 1. J. R. was a 32-year-old college student. On the first day the following results of blood studies were obtained: fasting blood sugar, 75 mgm. per cent; blood urea nitrogen, 17 mgm. per cent; carbon dioxide, 26 7 m.equiv./l.; chloride, 107 m.equiv./l.;

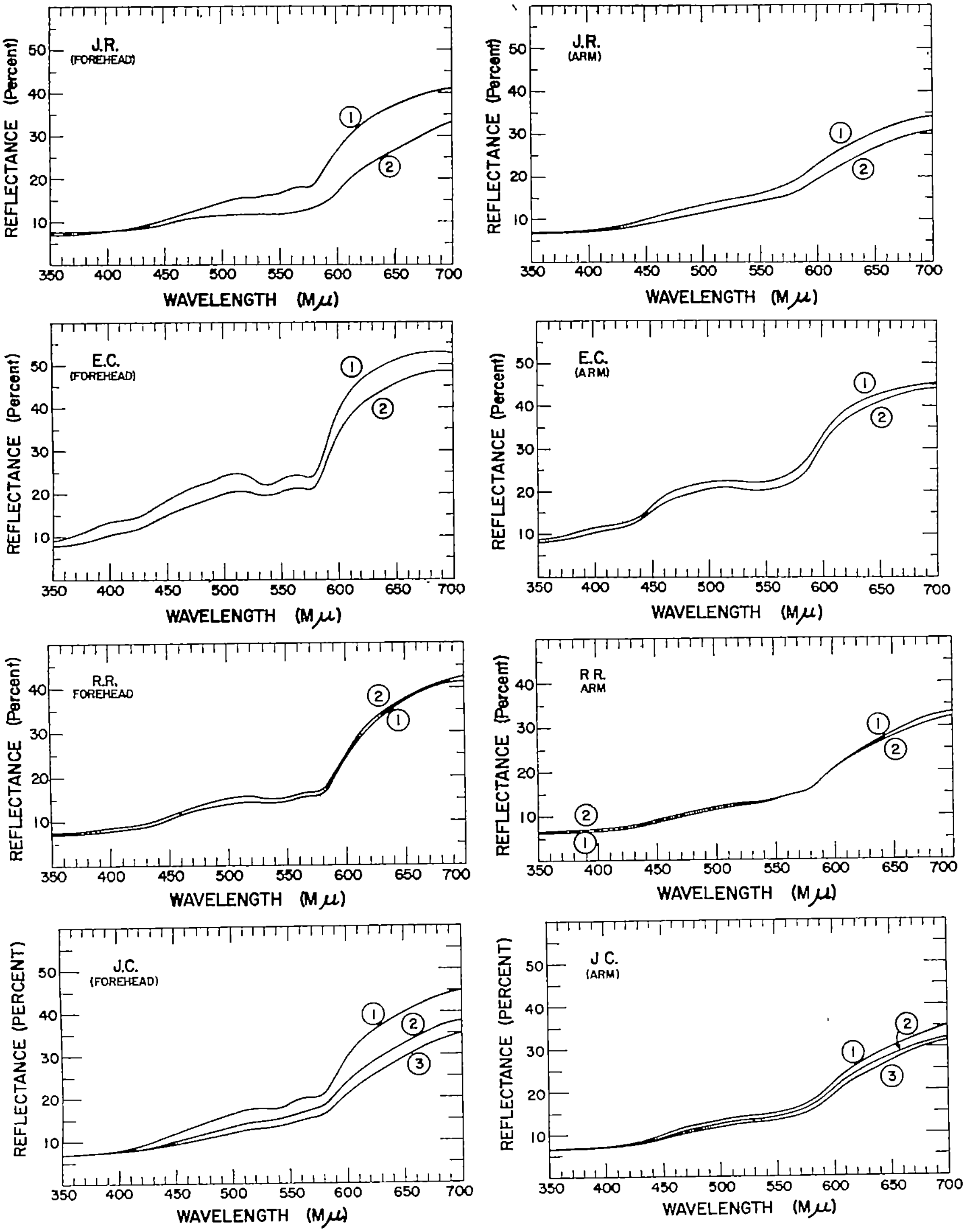


Fig. 2. Effect of melanocyte stimulating hormone on the reflectance of skin. The subject was seated beside a Cary model 14 reflectance spectrophotometer, and reflectance recordings were made of the extensor surface of the forearm and the mid-forehead, just above the glabella. The wave-length of incident light was varied from 700 mμ to 350 mμ. Duplicate tracings were made for each site
Subject J. R. Curve 1 was obtained before α-melanocyte stimulating hormone injections were given and curve 2 on the eleventh day of the experiment. The patient received on the first day, 3·1 mgm.; second day, 6 mgm.; third day, 9 mgm.; and up to the tenth day, 8 mgm. of α-melanocyte stimulating hormone a day. Note the decrease in reflectance throughout the spectrum
Subject E. C. received 8 mgm. of α-melanocyte stimulating hormone a day for ten days. Curve 1 was obtained on the first day and curve 2 on the thirteenth day. Note again the decrease in reflectance following administration of α-melanocyte stimulating hormone
Subject R. R. received 8 mgm. of α-melanocyte stimulating hormone a day for ten days. Curve 1 was obtained on the first day, curve 2 on the eleventh day. There was no change in reflectance during this period
Subject J. C. received 8 mgm. of β-melanocyte stimulating hormone a day for six days. Curves 1 and 2 were obtained on the first and seventh days. He then received 4 mgm. of α-melanocyte stimulating hormone for four days and 2 mgm. for one day. Curve 3 was obtained on the thirteenth day. There was definite darkening in this subject during administration of β-melanocyte stimulating hormone

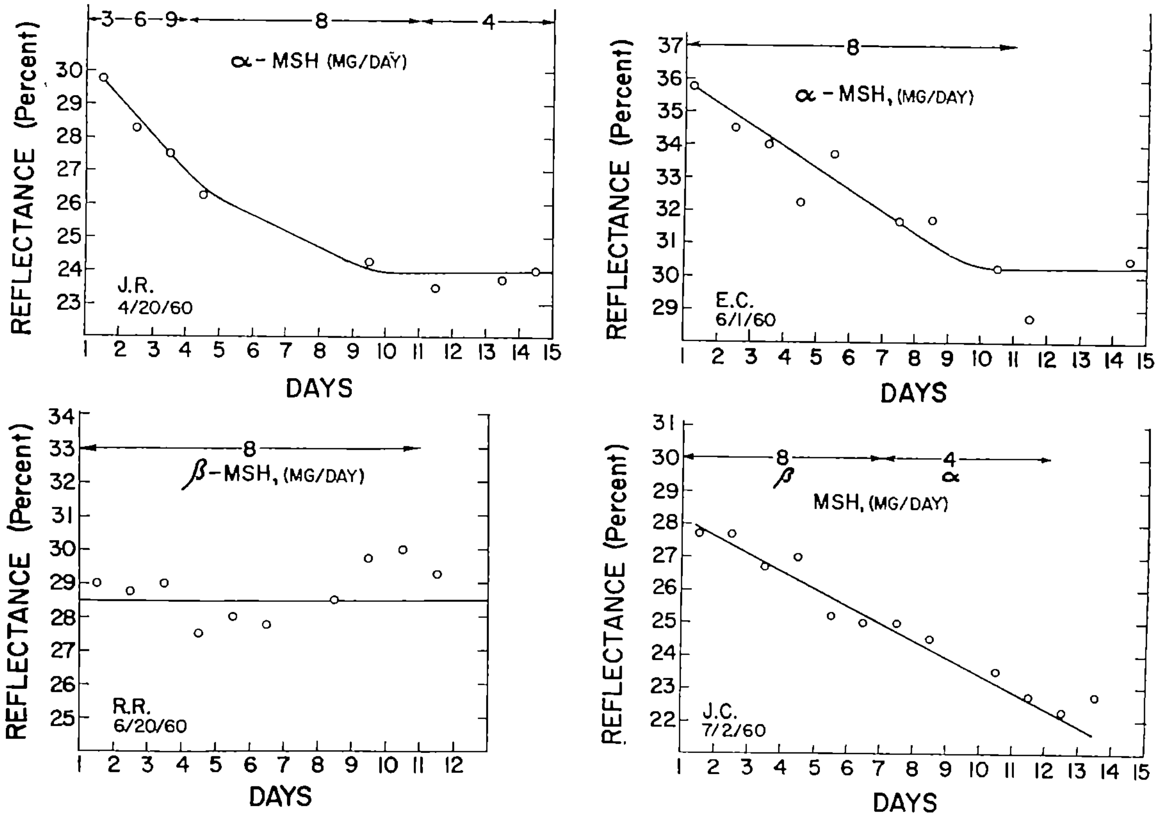


Fig. 3. Photovolt reflectance measurements during administration of melanocyte stimulating hormone. Reflectance of light from the skin was measured with a Photovolt model 610 photoelectric reflexion meter. The values used are arithmetic averages of separate readings made with red, blue, green and no filters. The forehead was measured in all patients except J. C., whose cheek was used. Here again it is seen that administration of α-melanocyte stimulating hormone to J. R. and E. C. resulted in a decrease in reflectance. Hog β-melanocyte stimulating hormone had no effect on R. R., but did cause a decrease in reflectance in subject J. C.

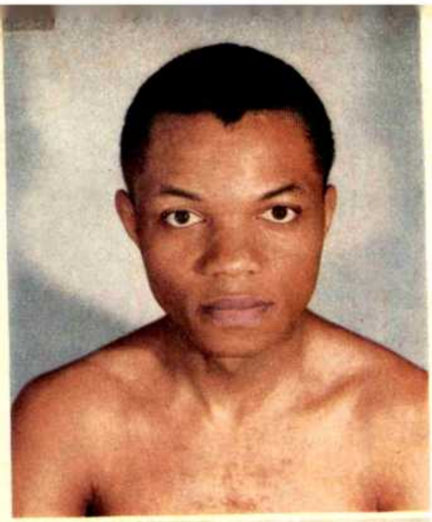
sodium, 141 m.equiv./l.; potassium, 4·7 m.equiv./l.; total protein, 8·5 gm. per cent; albumin, 3·2 gm. per cent; globulin, 5·3 gm. per cent; calcium, 12·1 mgm. per cent; phosphorus, 2·9 mgm. per cent; protein-bound iodine 7·8 μgm. per cent. On the fourteenth day these tests were repeated and found to be essentially unchanged: fasting blood sugar, 92 mgm. per cent; blood urea nitrogen, 12 mgm. per cent; carbon dioxide, 23·2 m.equiv./l.; chloride, 101 m.equiv./l.; sodium, 139 m.equiv./l.; potassium, 4·7 m.equiv./l.; total protein, 8 gm. per cent; albumin, 3·7 gm. per cent; globulin, 4·3 gm. per cent; calcium, 10·8 mgm. per cent; phosphorus, 3·4 mgm. per cent; protein-bound iodine, 7 μgm. per cent; erythrocyte sedimentation-rate, 7 mm./hr. The elevated globulin and calcium in the first group of studies suggested that the subject had sarcoidosis. However, X-ray examination of the chest was normal, and there were no symptoms or other signs of this disease. The activity of the synthetic α-melanocyte stimulating hormone was 3·3 × 10^{9} units/gm. On the first day 0·1 mgm. of melanocyte stimulating hormone was given intravenously over a period of 5 hr. in a litre of 5 per cent glucose in water. There were no changes in blood pressure or pulse, and three additional injections of 1 mgm. each were given that day. Six injections of 1 mgm. each were given on the second day. On the third day the patient and his wife noticed darkening of his face which they described as grey. On this day he received a total of 9 mgm. in seven injections. On the fourth day there were four injections of 2 mgm. each. This dose of 8 mgm./day was continued through the tenth day with two injections daily. Then 4 mgm./day was given in two daily injections of 2 mgm. each for four days. No melanocyte stimulating hormone was given after the fourteenth day.

The first increase in pigmentation appeared bilaterally in roughly circular areas just above the zygoma. The pigmentation was not even initially; but after an additional four or five days blended into the surrounding skin colour. Six weeks after the termination of the injections the subject had returned to his original colour.

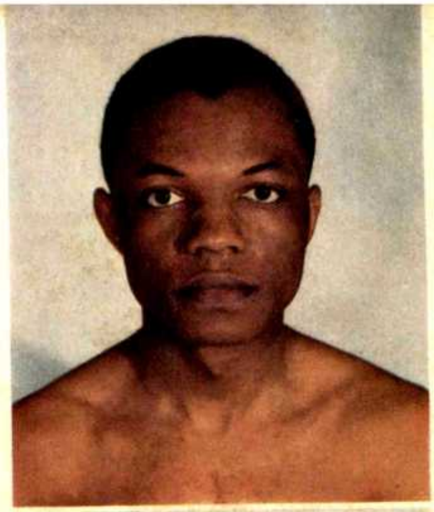
Subject 2. E. C., a 24-year-old hospital employee, was lighter than the first subject. His hyperpig-

Plate (facing page). Appearance before and after administration of melanocyte stimulating hormone. These photographs were made on Professional Ektachrome *E*-3, using an Eastman 81*A* colour correction filter. The lighting, shutter speed and diaphragm opening were not varied. The pictures of each subject were made on a separate roll of film. This helped eliminate variations due to developing. The photographs in all cases were made within 1 or 2 hr. of the Cary reflectance measurements in Fig. 2. The first picture was obtained just before treatment, the second picture just after treatment. The second picture of J. C. was made after β-melanocyte stimulating hormone and just before α-melanocyte stimulating hormone was given. There was darkening of both J. R. and E. C. following administration of synthetic α-melanocyte stimulating hormone R. R. did not darken after he received hog β-melanocyte stimulating hormone, but J. C. did darken

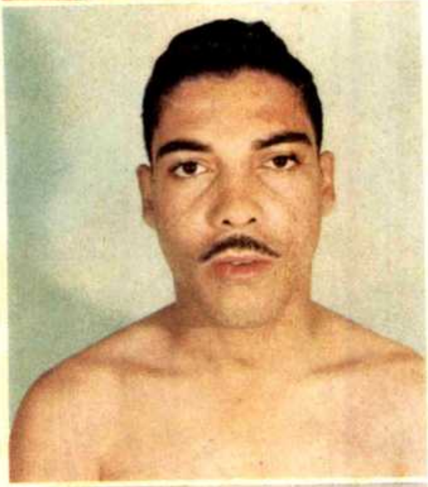
J. R. 1



J. R. 2



E. C. 1



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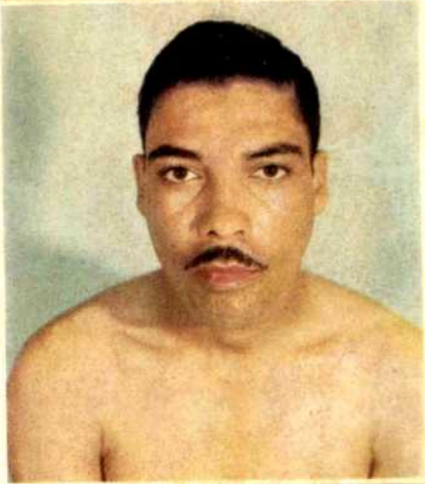

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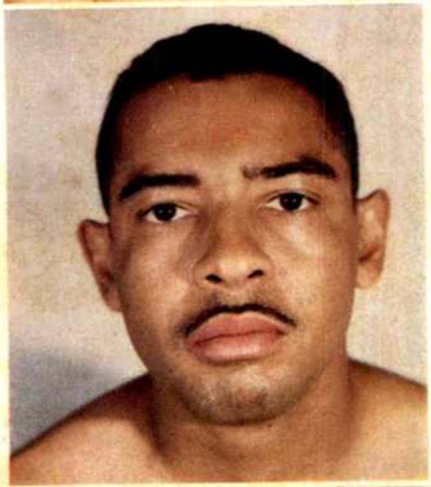

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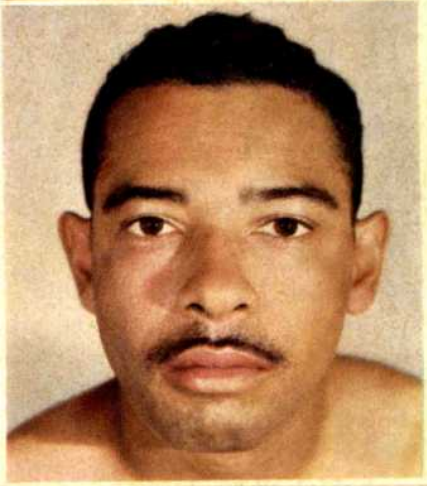

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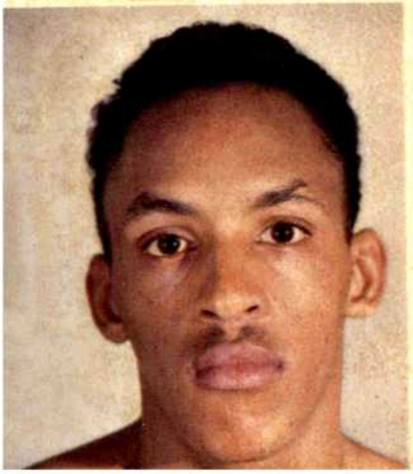

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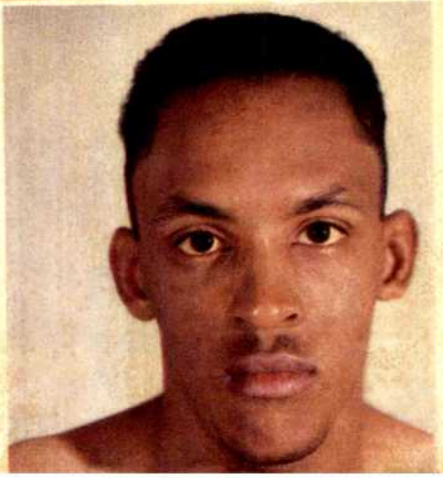
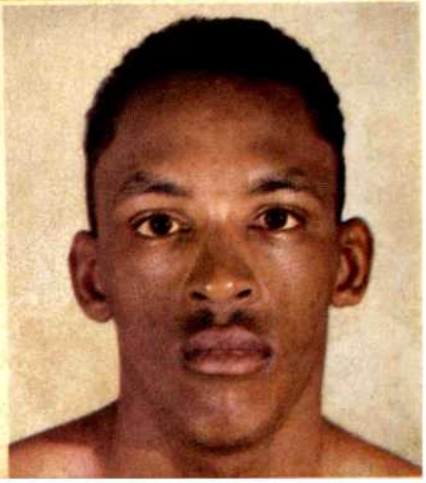


J. C. 3



mentation following the administration of melanocyte stimulating hormone was not so pronounced to the eye. He received 8 mgm./day of synthetic α-melanocyte stimulating hormone for ten days in two daily injections of 1·0 ml. of gel. The activity of the melanocyte stimulating hormone was $3\cdot3 \times 10^9$ units/gm. The melanocyte stimulating hormone used from the sixth to the tenth day had an activity of $2\cdot0 \times 10^9$ units/gm.

Subject 3. R. R., age 25, received, in two daily injections, 8 mgm. per day of β-melanocyte stimulating hormone with an activity of $1\cdot4 \times 10^9$ units/gm. for ten days. Routine analyses including blood count, urinalysis, blood urea nitrogen, fasting blood sugar, carbon dioxide, chloride, sodium, potassium, calcium, phosphorus and protein-bound iodine were normal on the first and last mornings of the test period. There was no observable or measurable darkening during the experiment.

Subject 4. J. C., age 23, received β-melanocyte stimulating hormone 8 mgm./day for six days in two divided doses daily. He darkened on this treatment. The β-melanocyte stimulating hormone had an activity of $1\cdot3 \times 10^9$ units/gm. To test the effectiveness in this subject of α-melanocyte stimulating hormone ($2\cdot0 \times 10^9$ units/gm.) which was more active than β-melanocyte stimulating hormone with regard to frog skin darkening, a smaller amount, 2 mgm./day of α-melanocyte stimulating hormone, was given for five days. There was some further darkening during this period.

It is interesting that the patients who darkened could detect the darkening at least as soon as we could measure it. This was attributed to the ability of the eye to integrate small changes in reflected light over a wide spectrum. The darkened skin had a decreased reflexion from 4000 to 7000 Å. Thus, melanin through its ability to absorb light of a large part of the spectrum is ideally suited for a light-protective role.

All subjects darkened more on the forehead than on the arm, and one (J. R.) showed quite striking darkening anterior to the ear above the zygoma. All hyperpigmentation had completely regressed within 6 weeks. The localization of the hyperpigmentation may result in part from the increased number of melanocytes present in the skin of the face[9].

From these experiments it is clear that systemically administered α-melanocyte stimulating hormone is an effective agent for darkening the skin of man. The relationship between melanin granule dispersion and new melanin formation in mammalian melanocytes is not known However, since darkening begins almost within 24 hr., it is possible that dispersion of melanin granules occurs within the melanocyte similar to that seen in the pigment cells of frogs and fish following exposure to melanocyte stimulating hormone. Newly synthesized melanin, however, may account for the prolonged hyperpigmentation.

β-Melanocyte stimulating hormone from hogs was not effective in the first subject, but was effective in the second one. Each subject received β-melanocyte stimulating hormone that had been prepared in different laboratories. Both preparations of β-melanocyte stimulating hormone had almost the same activity on frog skin. It is not known whether the difference in darkening of human skin was due to some unknown variation in the two β-melanocyte stimulating hormones or to a difference in response of the two subjects. Human β-melanocyte stimulating hormone was not available for testing. Neither α- nor β-melanocyte stimulating hormones had any effect on numerous metabolic tests carried out.

There is still doubt whether adrenocorticotrophic hormone can darken human skin. When this hormone was first introduced to clinical medicine approximately ten years ago, it was not uncommon to see patients develop pronounced hyperpigmentation following its use. These older preparations of adrenocorticotrophic hormone contained large amounts of melanocyte stimulating hormone[1]. More recently, commercial adrenocorticotrophic hormone has had much less melanocyte stimulating hormone, and the hyperpigmentation that occurs is less frequent and less pronounced. It would be possible to give 100–200 units of adrenocorticotrophic hormone free of melanocyte stimulating hormone to an experimental subject in order to test the effect of homogeneous adrenocorticotrophic hormone on skin darkening. With regard to darkening frog skin, this amount of adrenocorticotrophic hormone would be very small compared with the melanocyte stimulating hormone given the four subjects described in this report. On frog skin adrenocorticotrophic hormone has only one-thirtieth the activity of α-melanocyte stimulating hormone. Thus the amount of melanocyte stimulating hormone used daily in the present studies would equal more than 25,000 units of adrenocorticotrophic hormone in frog skin darkening activity. Perhaps some day it will be possible to give α-melanocyte stimulating hormone from which the N-terminal acetyl group is removed. The darkening action of this peptide might be like that of adrenocorticotrophic hormone which also has a free N-terminal serine.

One can ask whether the 4–8 mgm. of α-melanocyte stimulating hormone which was injected daily is a physiological amount of this hormone. Without knowing the amount of the injected material that is destroyed or sequestered by muscle and the amount normally produced by the human pituitary, this question cannot be resolved. All work carried out thus far has given results consistent with the view that the hyperpigmentation seen in patients with adrenocortical insufficiency and in some patients with pituitary tumours could be due to an increased release of α- or β-melanocyte stimulating hormone or both from the pituitary gland into the general circulation.

This work was supported by a grant from the U.S. Public Health Service, and during the tenure of a special fellowship from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service held by one of us (J. S. McG.).

We wish to thank Dr. M. Ruth Wright, who carried out the bioassay for α- and β-melanocyte stimulating hormones.

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POSSIBLE BIOLOGICAL EFFECTS OF ELECTRICALLY CHARGED PARTICLES IN TOBACCO SMOKE

By DR. K. H. KINGDON

General Electric Research Laboratory, Schenectady, New York

THE idea that inhaled atmospheric ionization may have some effect on humans has been current for about thirty years. Recent experiments by Krueger and Smith[1] have demonstrated that air ions incident on the mammalian trachea will increase or decrease the vibration frequency of the cilia according as the ions are negative or positive. I have pointed out[2] that 'glow discharge electrolysis' experiments[3,4] indicate that the ordinary 'small ions' of atmospheric electricity carry appreciable quantities of chemical and electrostatic energy (about 6 eV./ion), and I have found that 5×10^{-4} coulomb/sq. cm. of such ions incident on *Escherichia coli* will kill them to $1/e$. This amounts to 3×10^{8} ergs/gm. and is about eight times the amount of ultra-violet energy or 700 times the amount of X-ray energy required to kill *E. coli*.

With the establishment of definite biological effects of atmospheric small ions such as those referred to above, it becomes of interest to inquire whether the ions which are well known to be present in tobacco smoke are of such a kind, or are present in sufficient numbers, as to produce significant biological effects when inhaled.

Ions in Smoke from Tip of Cigarette

Ions from the cigarette were analysed by drawing them in a current of air through the standard mobility equipment of atmospheric electricity, the particular apparatus being operated so as to collect small ions of mobility greater than 0·4 cm.^{2}/sec. volt and to reject slower ions. The sum of large and small ions was measured, without analysis, by other equipment. Air was drawn through the cigarette as needed with equipment giving a 'standard draught' of 35 c.c. in 2 sec. The ambient air currents at the cigarette tip were gentle enough so that after initial ignition a 'king-size' cigarette required 14 min. to burn up, without further 'standard draughts'. The ions are presumably generated by thermal ionization of potassium, which is always present in substantial amounts.

The first attempts to observe small-ion currents from a burning cigarette showed that small ions were indeed generated, but in numbers which increased greatly with application of a few hundred volts, or more, to the mouth end of the cigarette. The applied voltage creates a field at the burning tip which separates the positive and negative ions generated there and prevents their rapid recombination. Simple measurements show that a smoker's ordinary movements will raise his body to potentials of hundreds of volts, so that an adequate voltage is often applied automatically to the cigarette.

Fig. 1 shows the small-ion current as a function of voltage applied to the mouth end of a burning cigarette. At a few hundred volts the current is of the order of 10^{-10} to 10^{-9} amp. The current increases systematically with voltage, but shows a marked saturation trend above 1,000 V.

In addition to the emission of small ions, there is an emission of large ions which is about an order of magnitude greater than the small-ion current. The currents of both large ions and small ions increase sharply when the temperature of the cigarette is raised by giving it a standard draught. Both currents also increase sharply when ash is removed so as to expose the burning area.

The saturation behaviour of the small-ion current is due to the high electrical resistance of the cigarette. It was found that the resistances of a number of unlighted cigarettes were of the order 10^{10}–10^{11} ohms, and that although the resistance of the cigarette was lowered temporarily by taking a standard draught, the resistance returned to a high value between draughts.

Fig. 2 shows the variation with time of the emission of small ions from a cigarette charged to 1,000 V. after one standard draught only. The emission fluctuates down and up as ash accumulates or is removed by natural fall and by tapping. It is clear that the cigarette is a continuous source of small ions of the order 3×10^{-10} amp.

Ions are present in the smoke drawn through the cigarette and increase in number as the electric field

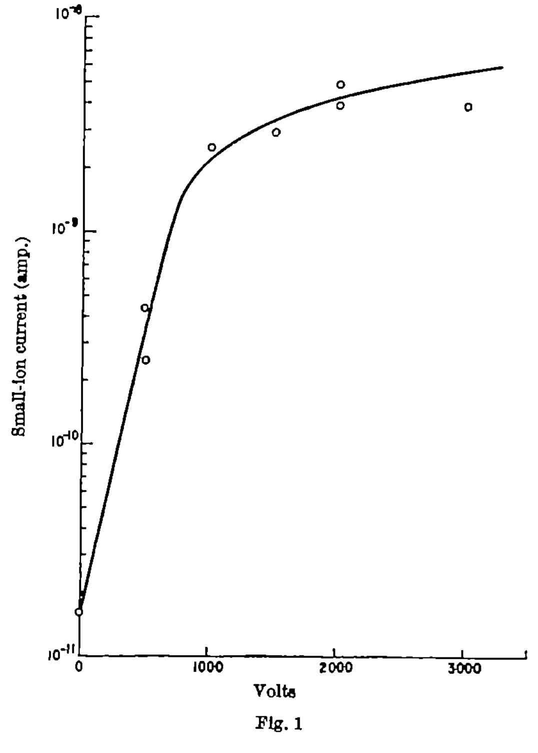

Fig. 1

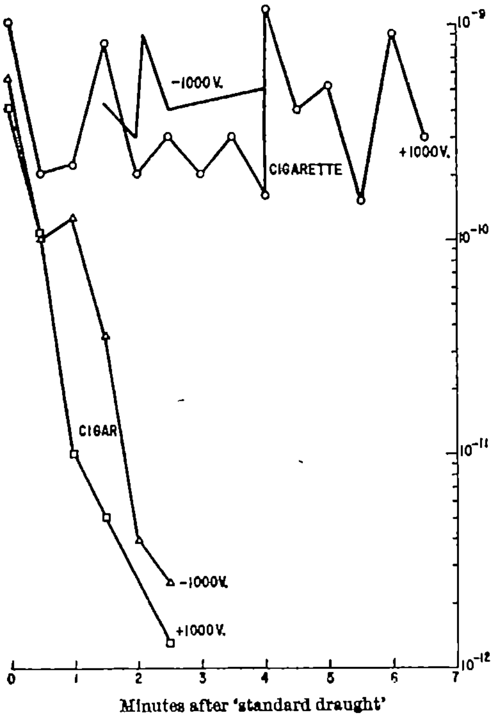

Fig. 2

at the burning tip is increased. No small ions could be detected in this smoke stream. In a more rigorous test of this sort, small ions generated by electrical corona were used. It was found that passing this ion stream through a 1-cm. length of cigarette reduced the small-ion content to 1/3,400, and passing it through 0·7 cm. of filter tip reduced the small-ion content to 1/8,500. The conclusion from these tests is that any small ions generated at the burning tip are removed (to about 1/10,000) by Brownian movement and electrical forces on their way through the cigarette and do not enter the mouth. The large ions, formed by coalescence of small ions with smoke particles, are thought not to have any specialized biological effect ascribable to their charge[3].

Unipolar small ions leaving the tip of a charged cigarette are spread throughout a substantial volume by thermal or air current convection, by motion of the cigarette, and by their mutual repulsion. The repulsion limits the concentration of unipolar ions which can be built up in the vicinity of the cigarette. Thus if N positive ions of charge e are within a sphere of radius 25 cm., the electric field Ne/r^2 at the surface of the sphere for $N = 1 \cdot 8 \times 10^{10}$ will be 4 V./cm. The ions at the surface of the sphere will move outwards with a velocity of 4 cm./sec., about twice that given to them by the ordinary atmospheric electric field. The average concentration in the sphere of radius 25 cm. is $2 \cdot 8 \times 10^5$ ions/c.c. It appears that the generation of small ions by the cigarette can maintain a concentration of this order, but it also appears that repulsive forces will prevent the concentration from being much higher.

It seems likely, therefore, that a smoker inhales small ions at a concentration of approximately 2×10^5 ions/c.c. all the time that he is smoking a cigarette. This concentration is about one thousand times that found in an ordinary city bedroom with no smoking going on.

Similarities between Experimental Small-Ion Effects and Alleged Correlations of Smoking with Lung Cancer

The discussion so far has dealt with the physical characteristics of the emission of small ions from a burning cigarette. The remainder of this article will be devoted to an exploration of the possible relation between continued inhalation of super-normal quantities of small ions and the development of lung cancer. This hypothesis is envisaged as an alternative to the concept that lung cancer may be caused by carcinogenic chemicals in tobacco smoke. The carcinogenic chemical concept is not universally accepted[5,6] as a major cause of lung cancer, so it seems worth while to explore an alternative. Explanations offered by this alternative hypothesis for three areas of observation in smoking and lung cancer will be compared first.

Comparison of cigarette, cigar and pipe smoking. Evidence has been presented in the literature which is alleged to show that lung cancer is much less frequent among cigar and pipe smokers than it is among cigarette smokers. Data have been presented in the present article to show that a charged, burning cigarette is a relatively strong and continuous source of small ions. Similar tests with a cigar showed that although a vigorously smoked, charged cigar (two successive standard draughts) gave transient small-ion currents comparable with those from a cigarette (see Fig. 2), the current decreased rapidly with time, so that a cigar is a much weaker continuous source of small ions than a cigarette. Similar tests with a charged pipe showed it to be an even weaker source.

Alleged differences between the behaviour of cigarettes, cigars and pipes in producing lung cancer may thus be correlated very simply with observed differences in the numbers of small ions produced.

Site of lung cancer as observed in diagnosis. The type of lung cancer which is alleged to be connected with smoking is observed first in the lower parts of the bronchi. Particles capable of rapid diffusion, such as the small ions, are much more likely to be deposited in the bronchi than are the slow, 0·5 micron droplets, carrying the alleged carcinogenic chemicals.

Failure to give mice lung cancer by inhalation of smoke from a smoking machine. Several attempts have been made to develop lung cancer in mice by exposure to smoke from a smoking machine[7], but in such experiments the development of cancer has not exceeded the normal expectancy. It has been pointed out above that by passage through the cigarette small ions are completely removed from the smoke of even a strongly electrically charged cigarette, so that the mice, although possibly exposed to inhalation of carcinogenic chemicals in the smoke, were not exposed to the effects of any small ions which may have been created initially in the smoke.

Possibility of Biological Damage from Inhalation of Small Ions

Data are not available to permit a convincing calculation of the possibility of lung damage resulting from inhalation of small ions in cigarette smoke. However, it is worth noting that the biological effect of small ions is closely similar[3] in most details to the familiar indirect X-ray process of radiation biology. Both processes involve the initial formation of a positive ion and a free electron, which afterwards give rise to active chemical species. The chemical energy of these secondary products causes the final biological effect. In view of the major similarity between the two processes, it seems reasonable to put forward as a hypothesis that if prolonged exposure to a low intensity of X-rays or other ionizing radiation can cause lung cancer, prolonged exposure to small ions at an intensity about one hundred times normal may also cause lung cancer.

It should be emphasized, however, that as yet there is no experimental demonstration of this, and the small-ion hypothesis is simply advanced as an alternative approach which appears to merit further exploration.

The emission of small ions from a charged, burning cigarette can be completely suppressed by smoking the cigarette within a screen which always encloses the burning tip and maintains zero field there. Suppression of emission of small ions is complete even when cigarette and shield are charged to 3,000 V. If further experimentation confirms the hypothesis of the production of lung cancer by small ions, it would be very satisfactory to have available such a simple and effective means of suppressing the effect.

Since potassium, or some other element of low ionization potential, is probably intimately connected with the formation of the small ions by thermal ionization, elimination of this element from tobacco presumably would reduce greatly the generation of small ions from an unshielded cigarette.

I am indebted to the General Electric Research Laboratory for making laboratory facilities and equipment available for carrying out the work just after my retirement. I wish also to express my appreciation to C. M. Bishop for continued help with the experiments, and to H. Hurwitz, jun., J. L. Lawson and J. K. Bragg for constructive criticism.

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CENTENARIES

By JOAN M. EYLES

Samuel Clegg, 1781–1861

SAMUEL CLEGG the elder, a pioneer gas engineer, died at Haverstock Hill, Middlesex, on January 8, 1861, within two months of his eightieth birthday. Clegg was born in Manchester and received some of his education from John Dalton, who was teaching in Manchester from 1793 onwards. About this time William Murdock was carrying out his first experiments in gas-lighting while he was employed by the Birmingham firm of Boulton, Watt and Co. When young Clegg later became apprenticed to this firm, they were developing their first gas-making plants. It is not surprising, therefore, that his interests turned in that direction.

He left the firm in 1805 to set up in business himself, and soon after installed gas-lighting in the cotton mill of Henry Lodge at Sowerby Bridge, near Halifax. In 1808 he was awarded a silver medal by the Society for the Encouragement of the Arts for his apparatus for making gas from pit coal and lighting factories therewith. Later on he moved to London, and in 1812 installed gas-lighting in both the dwelling-house and business establishment of Rudolph Ackermann, the well-known art publisher, at 101 Strand.

In 1812 the Gas, Light and Coke Company obtained a charter to operate in the metropolis, and a few months later Clegg became its chief engineer. He was with the Company for nearly five years, and undoubtedly its success owed much to his ability. Clegg was responsible for the lighting of Westminster Bridge in 1813 (even lighting the lamps himself when the lamplighters refused to risk it) and also for the installation of gas mains along many of the streets of Westminster and the City. A notable achievement was the lighting of Guildhall in the City, inaugurated on November 9, 1815. During this period he introduced many improvements, including the first gas-meter and a gas governor; he also designed a horizontal rotary retort, which was incorporated in the plant installed at the Royal Mint by F. Accum, in 1817, the year in which Clegg left the Gas, Light and Coke Company.

Clegg afterwards suffered financial reverses. He worked for a time as engineer to the Portuguese Government, and also became involved with a project for a so-called atmospheric railway, in which a vehicle attached to a piston in a continuous pipe is made to move by maintaining a low pressure on one side of the piston. Later, however, he received a Government appointment as a gas surveyor, and also assisted his son, Samuel Clegg the younger, with the preparation of a treatise on the manufacture of coal gas.

Sir James Hall (1761–1832)

SIR JAMES HALL of Dunglass, fourth baronet, was born on January 17, 1761. He succeeded to the title when only fifteen, on the death of his father, Sir John Hall. In 1784 he was elected a member of the recently founded Royal Society of Edinburgh, a society with which he was closely connected for the rest of his life, publishing his scientific and literary papers in its *Transactions*, and holding office as president during 1812–20.

When Hutton's famous paper on a "Theory of the Earth" was read to the Society in 1785, Hall was abroad, studying among other matters the lavas of Vesuvius. At that time he was not attached to any system of geology, but later was won over to the Huttonian system and then became one of its most ardent supporters. He saw the possibility of testing the validity of some of Hutton's ideas by laboratory experiment, but this suggestion was not welcomed by Hutton himself; and it was only after the latter's death in 1797 that Hall began to carry out experiments seriously and systematically.

In 1798 he showed that glassy material obtained by heating strongly different lavas and whinstones would revert to a crystalline material if cooling was sufficiently slow. He followed these experiments with others designed to show the "Effects of Compression in modifying the Action of Heat", in which his aim was to demonstrate that when powdered limestone was strongly heated it would, if heated in a confined space, fuse and re-crystallize instead of decomposing. Using gun-barrels and other apparatus of his own design, he succeeded in his objective, obtaining results on which subsequent experimenters were unable to improve substantially for nearly a century, and which clearly show his skill and perseverance at a time when pyrometry and furnace control were in their infancy. Indeed, he opened up an entirely new field of research in chemistry and geology.

Hall's interest in experimental geology continued through his lifetime, and he investigated the effect of lateral compression on bedded strata, and the causes of consolidation of strata. After his death in 1832 some of his apparatus and a number of specimens were presented to the Geological Society of London by his son, Captain Basil Hall, and they are now in the collections of the Geological Museum.

OBITUARIES

Prof. G. W. O. Howe

The initials G.W.O.H., familiar to radio engineers throughout the world, passed from currency on November 7 with the death of Prof. George Walter Osborne Howe.

Howe, who was born on December 4, 1875, at Charlton, Kent, and educated at the Roan School, Greenwich, took pride in the long association of his family with riverside London. His forebears were, he used to say, Thames watermen.

After an engineering apprenticeship with Siemens Brothers in Woolwich, Howe became an undergraduate at Armstrong College, Newcastle on Tyne. He graduated with honours from the University of Durham in 1900 and he had the distinction of being the senior Whitworth Scholar in that year.

During 1900–02 he obtained experience in the design of electrical machines at the Siemens-Schuckert works in Berlin, this incidentally being a period in which the German designers of electrical machines were pre-eminent in Europe.

On his return to England, he took up his first teaching appointment in the Hull Technical College, and apart from a brief interlude in 1920–21 at the National Physical Laboratory, where he was head of the Department of Electrical Standards and Measurements, the whole of his long career was devoted to academic work.

It was in 1905 that he was appointed to a lectureship in the City and Guilds College, and there he began to make his major contributions to electrical science. His most notable original work was on radio propagation, the behaviour of radio aerials and on the penetration of high-frequency currents into conductors. His earliest papers show that distinctive quality of mind which was to characterize all his publications and to bring to him in later years a reputation which was truly world wide. His intellectual armoury comprised a superb knowledge of classical electromagnetism, exceptional powers of physical visualization, and the capacity to devise new methods of approach to complex phenomena whereby familiar concepts and relatively simple mathematical methods could be applied.

During 1910–20 he contributed to *The Electrician* a series of papers dealing in the main with high-frequency phenomena, and he made communications on the same subject to the *Proceedings of the Royal Society* and to the *Journal of the Institution of Electrical Engineers*.

In 1921 he was appointed to the newly created James Watt chair of electrical engineering in the University of Glasgow. At about the same time the monthly journal *Experimental Wireless*, now *Electronic Technology*, invited Prof. Howe to become its technical editor. This editorship, which lasted for more than twenty-five years, must be one of the most remarkable in the history of scientific publishing. Every month an editorial appeared over the initials G. W. O. H. Just occasionally the editorial would be of conventional form, commenting in a general way on some development or perhaps on an international conference. The regular pattern was, however, a closely reasoned exposition of some piece of electrical theory relating to a topic of current interest. The article might expose a fallacy, might derive by a brilliant simplification a result obtained in some recently published paper by a recondite method, or might treat a difficulty which had arisen in the course of discussion with his colleagues. Many of the problems which he tackled involved conceptual difficulties of a high order and he would admit to many hours of anxious thought over some of them and, on one or two occasions, to momentary misgivings after he had gone to press. In these editorials, not only was his capacity for clarity of exposition displayed at its best, but also his genius for the apt simile and for witty and sometimes caustic comment had free play. Muddled thinking or obfuscation would sometimes provoke him to a sharpness of witty sarcasm which could give a false impression of him.

Quite early in his career he began attending meetings of the British Association. He became, in due course, secretary and afterwards recorder of Section G (Engineering), and in 1924 president of that Section. He attended three of the meetings which the British Association held overseas—Adelaide in 1914, Toronto in 1924 and Johannesburg in 1929. In January 1938 he went as a member of the official delegation of the British Association to the jubilee meeting of the Indian Science Congress which was held in Calcutta.

During the later part of his life he maintained a direct association with the radio-manufacturing industry as a director of the Mullard Radio Valve Company.

Prof. Howe was elected to associate membership of the Institution of Electrical Engineers in 1905. He took a prominent part in the formation of the Wireless Section of the Institution in 1919 and he was elected chairman of the Section in 1921. In 1956 the Institution awarded to Prof. Howe the Faraday Medal in recognition of his pioneering work in the study and analysis of high-frequency oscillations and of the theory of radio propagation and for his outstanding contributions to engineering education. The Institute of Radio Engineers of New York in 1955 also conferred on him the high honour of election to fellowship of the Institute.

His scientific work apart, Howe experienced his greatest enjoyment in his music. He was an accomplished violinist. He had been taught, he said, when very young on a miniature violin specially made for him, and he could not remember a time when he did not play the instrument. Until a year or two before his death he was an active member of the Glasgow University Orchestral Society.

Only those who had the good fortune to study under him knew the quality of his teaching. He was one of the greatest teachers of his generation. It is hard to escape the conclusion that the main driving force in his scientific work was the urge first to understand, in Clerk Maxwell's boyish phrase, "the go of the thing" and then to expound it. His mind was essentially scholarly, and yet one got the impression that he was intrigued by the unexpected practical implication, the apparent anomaly or the amusingly odd concept rather than by the deeper philosophical implications.

A lively and entertaining companion, a delightful host and an equally delightful guest, Prof. Howe had a wide circle of friends. It was only rarely that the brilliant mind, which in its day-to-day contacts with the world gave the impression of being quietly but confidently self-contained, revealed for a moment that depth of human feeling of which it was capable.

Prof. Howe married in 1901 Sarah Alice Maunder. Mrs. Howe died in 1930. J. GREIG

Dr. Norman Bauer

NOT often is exceptional scientific ability and a deep concern for the welfare of his fellow man combined in one individual. Such an individual was Dr. Norman Bauer.

Dr. Bauer began his studies at the University of California at Berkeley, graduating with a B.S. in chemistry in 1937. He then entered the University of Michigan, receiving his M.S. in 1939 and his Ph.D. in 1941, under the direction of Prof. K. Fajans. He held a scholarship during 1936–37 at the University of California and was Eastman Kodak Fellow at the University of Michigan during 1940–41, and a Post-doctoral Fellow at that institution during 1941–42.

Desiring to teach, he joined the faculty of the University of New Hampshire in 1942 and remained there until 1945. During this period he was co-author with Prof. Fajans of the sections on the determination of density and refractometry in Weissberger's *Technique of Organic Chemistry*, having done much important research in these fields. Due to personal obligations, he returned to California in 1945, where he was employed by the California Research Corporation. Here he became interested in mass spectrometry, publishing articles in this field. In 1949 he founded an independent research laboratory under the name Physical Science Associates at Berkeley. Later, with his personal obligations changed, he returned to academic life, accepting a position at Utah State University in 1953, where he was most recently professor of chemistry.

Here he carried on an excellent programme of teaching and basic research in physical chemistry. His knowledge and enthusiasm encouraged research in several fields, and many new methods of approaching problems in soil and plant nutrition and in the thermodynamics of the water relations of soils and plants were developed by him. He became interested in the problem of biological nitrogen fixation and made substantial progress in this difficult field, studying the physical chemistry of legoglobins (the proteins involved in nitrogen fixation), establishing a reputation for his laboratory in this topic. Recently, he made significant discoveries that may lead to a major advance in the solution of this problem. Working with electron spin resonance equipment at Varian Laboratories during the summer of 1960, he detected the presence of free radicals that could be key intermediates in nitrogen fixation. This was especially gratifying to him, since he had predicted theoretically the existence of such radicals in a study of possible pathways for the process. His death on September 9 prevented him from following up this important possibility.

Dr. Bauer was concerned about many problems involving man's welfare. He was opposed to further testing of atomic bombs and personally paid for advertising space to register his protest. He published articles questioning the reasoning of the U.S. Atomic Energy Commission on the hazards of radioactive fall-out. He was particularly concerned with the dangers of nuclear war and spoke out strongly for the cause of peace. He was a champion of the cause of academic freedom, taking an active part in upholding the right of free inquiry. A dedicated scientist, a gifted teacher, he was, in every sense, a man of unquestioned integrity and morals who brought honour to his profession, and a man of good will. JACK T. SPENCE

Mr. Jaques Cattell

JAQUES CATTELL, editor of *American Men of Science*, publisher of the *American Naturalist*, and president of the Jaques Cattell Press, Inc., died after a long illness at New York Hospital on December 18: he was fifty-six years old.

In 1923 Mr. Jaques Cattell became associate editor of *American Men of Science* with his father, the late Dr. James McKeen Cattell, and editor following the death of his father. He was also editor of *Leaders in Education*, founded by his father. The *Directory of American Scholars* was established by him in 1942, under the sponsorship of the American Council of Learned Societies.

Mr. Jaques Cattell was interested in scientific publishing and the graphic arts. He was president of Science Press Printing Company, and later was a pioneer in the use of cold type composition in scientific publishing. In 1959 he moved to Arizona, and, at the invitation of the President of Arizona State University, continued the publication of his biographical directories from the University campus in Tempe.

He is survived by his widow, the former Elizabeth Walsh, and his son, James Cattell.

The Jaques Cattell Press, Inc., has announced the appointment of Garrison Cattell and Dorothy Hancock as acting editors of *American Men of Science*. Mr. Cattell and Mrs. Hancock have worked closely with Mr. Jaques Cattell in editing and publishing this directory.

NEWS and VIEWS

Mathematics at Canberra:

Dr. B. H. Neumann, F.R.S.

Dr. B. H. Neumann has been appointed to the newly established chair of mathematics at the National University of Australia, Canberra. Bernhard H. Neumann was born in Berlin in 1909. He received his early academic training at the Universities of Freiburg and Berlin, where he obtained the degree of Dr. phil. in 1932. Soon afterwards he left Hitler's Germany and continued his research work at Cambridge, where he graduated Ph.D. in 1935. His first teaching appointment was at University College, Cardiff (1937–40). This was followed by war service from 1940 until 1945. After the War he joined the staff at University College, Hull (1946–48). Since 1948, Dr. Neumann has been a member of the Mathematics Department at Manchester, first as a senior lecturer and later as a reader. Neumann's published work is almost exclusively devoted to the theory of groups. He has enriched this important branch of modern algebra by outstanding contributions published in numerous articles, in which he lucidly expounds his results, solves old problems and poses new ones. His achievements have been recognized by academic distinctions which include the Adams Prize (1951–52), the D.Sc. degree at the University of Manchester (1954) and the election to the Royal Society (1959). Besides his creative powers, Dr. Neumann possesses a remarkable talent to inspire young mathematicians and to guide them through all the stages of their career as research workers. He will no doubt attract to Canberra a team of enthusiastic collaborators. Chief among these will be his wife, Dr. Hanna Neumann, who is well known for her own work on group theory.

Engineering Science at Oxford:

Prof. A. Thom

Prof. A. Thom is to retire from the chair of engineering science at the University of Oxford, which he has held since 1945. Prof. Thom is an engineer with an aeronautical bias, who worked in the Royal Aircraft Establishment, Farnborough, during the whole of the Second World War, after a long period as a lecturer at the University of Glasgow. He is best known for his skill and inventiveness in the field of numerical analysis by desk computers. In particular, he has obtained a large number of useful results in theoretical fluid dynamics and other subjects by his 'squares' method, an alternative to Southwell's 'relaxation' approach. He is also well known for a number of important wind-tunnel investigations.

Prof. D. W. Holder

Dr. D. W. Holder, who is to succeed Prof. Thom in the chair of engineering science at the University of Oxford, is like him in that he is an aeronautical engineer, who has built up since the War a remarkable research team in high-speed aerodynamics at the National Physical Laboratory. This team has been responsible for a large proportion of the fundamental advances in transonic, supersonic and hypersonic gas dynamics in Britain since 1945. The research facilities which he has built up have been truly impressive, but his contributions towards interpretation of the results have been equally important, notably in the field of shock-wave boundary-layer interaction.

Current Research and Development in Scientific Documentation

A recent issue of *Current Research and Development in Scientific Documentation*, issued by the National Science Foundation, lists 159 research projects in 99 organizations, compared with 61 in 39 organizations a year ago (No. 7. Pp. viii+153. Washington, D.C.: Government Printing Office, 1960. 65 cents). Of these 17 are listed under information requirements and uses; 45 under information storage and retrieval; 26 under mechanical translation; 17 under equipment; and 54 under potentially related research which is not directly connected with scientific documentation, but which may influence such work in the future. The present issue is provided with a detailed subject index and an introductory statement to each section directing attention to new work and to closely related projects reported in other sections. In the first section may be noted the study by the International Federation of Documentation of the availability and value of scientific conference papers and proceedings; a study of the availability and publication of scientific information contained in unclassified Government research reports; studies of the use of photocomposition and of microform publication, of the information needs of scientists in the field of psychopharmacology and of the need for an abstracting service in soil and water technology. In information storage and retrieval most of the effort is concentrated on systems design, but there is growing interest in evaluation and comparison of the newer systems, in more theoretical work on systems design and in mechanized processing of complete texts. Mechanical translation research up to the present has been mainly concentrated on the problems of lexicon and the passing of the source languages, principally Russian and English, with relatively little attention to the synthesis of the output language. The most noticeable trends are the increasing number of languages under study and the increasing breadth and generality of the theoretical work. Under equipment, particular emphasis is being placed on the development of storage devices and of output components.

Education and Industry

In an article that appeared in a recent issue of *ESSO Magazine* (9, No. 4, Autumn, 1960), Sir Hugh Beaver suggests that people in Great Britain can no longer afford to argue about education for management, and that it is no longer realistic to call on "industry to exercise the greatest care in recruiting graduates", in order to allow the schools to have a proportion of the 'good people' for advanced studies. He argues that to many, what matters are the financial attractions, and says that no one should question or deplore such an attitude in any individual, now that it has been shown that industry offers as great an opportunity of real service to the community and to the country as any of the professions. There are many thousands of companies in industry and commerce who want an educated intake but who are harassed by the deficit due to a shortage of teachers. Such difficulties could be solved, he suggests, by a system of senior part-time appointments or temporary secondments, or by alleviating the problem of the shortage of teachers itself. However, he directs attention to the fact that it cannot be claimed that we are deploying Britain's full resources so long as women play such a small part in our industrial, scientific, technological and high-management activities. Sir Hugh also touches on the increasing specialization which now occurs right through industry. These specializations are now acceptable, and the formal ladder of promotion and success must be by one or other specialization. The great task that confronts all who are training managers is to produce people who, while initially master of one or other specialization, can at the same time understand how the other parallel and complementary specializations all build the one body, whether it is their own company or the industry of which their company is a unit. Only by such means will they be capable of becoming managers with wide responsibilities.

Mental Health

DURING 1959–60 the major concern of the National Association for Mental Health has been with the promotion of World Mental Health Year in the United Kingdom and with preparations for the coming of the Mental Health Act. These are described in the fourteenth annual report (Pp. 55. London: National Association for Mental Health, 1960). Before the Mental Health Act received the Royal Assent on July 29, 1959, the Ministry of Health had issued circulars in anticipation of its coming. The most noticeable was that issued during January 1958, authorizing the informal admission of patients to mental deficiency hospitals. Following the passing of the Mental Health Act, the Minister of Health issued a direction to local authorities asking them to submit details of their proposals for community care. These are now being studied at the Ministry. Another development of considerable importance to the Association is the initiative of the Royal College of Nursing in petitioning the Privy Council for an alteration in its Charter to enable all registered nurses to become members of the College. If the petition is granted, mental nurses, male and female, may, for the first time, become members of the Royal College of Nursing. Information about the work of the Association can be obtained from Maurice Craig House, 39 Queen Anne Street, London, W.1.

Polish Scientific Publications

THE *Quarterly Review of Publications*, published since 1955, describes publications of the Polish Academy of Sciences and of such scientific societies as are supported by the Academy. For the convenience of readers interested in particular disciplines, the *Review* has appeared since 1958 in three series: Series *A*, Social Sciences; Series *B*, Biological Sciences; and Series *C*, Pure and Technical Sciences. To give the reader a more complete picture of current Polish scientific literature, the editors have decided to include in the *Review* the more important scientific publications of institutions other than the Polish Academy of Sciences and the affiliated societies. Each entry published in the *Review* is supplemented by a brief summary. Titles are quoted in the language used by the author and, except in the case of Latin and Greek titles, an English translation is given. Copies of the *Review* can be obtained from the Distribution Centre, Warszawa, Palac Kultury, 1, Nauki.

Peabody Museum of Yale University

THE annual report of the Peabody Museum of Yale University for 1960 is unusual in that it is the first to be issued by the Museum and is also the first by the present director, Mr. S. Dillon Ripley (Pp. 31. New Haven, Conn.: Peabody Museum of Natural History, Yale University, 1960). It also coincides with the re-activation of the Museum Trustees and the Advisory Committee. The report records the opening of the new wing on October 30, 1959, which event marked the culmination of nearly 15 years planning. The director rightly states that the health of a museum depends not only on the development of exhibits, or the active progress of school services, but also on the calibre of the research by the staff and those associated with it. An impressive list of publications is a measure of the research carried out by this Museum. It is intended that reports should include semi-popular articles on natural history subjects—the first one being on the wren.

The Australian Museum

THE first part (two stories) of the new six-storey wing of the Australian Museum was officially opened by the Premier of New South Wales, Mr. R. J. Heffron, on August 11. The completion of this first part is one of the outstanding events in the history of the Museum, for it is the first major building addition for half a century. The new work is faced with handsome sandstone and contains curatorial offices, laboratories and space for reserve collections. The contract for the erection of the other four floors has already been signed and these will enable further collections to be displayed and also provide for a public cafeteria on the roof.

Impact of *Lunik* 2 on the Moon

AT about the time when *Lunik* 2 struck the Moon, 21h. 02m. 24s. U.T. on September 13, 1959, optical observers in several parts of Europe noted small disturbances on the Moon's surface. Their observations, none of which could be certainly linked with *Lunik* 2, were reviewed by G. Fielder (see *Nature*, 185, 11; 1960). One group of observations, from Hungary, has now been described in more detail by L. Detre, in a publication entitled "Bericht über Optische Beobachtungen Anlässlich der Landung der Sowjetischen Mondrakete Lunik II" (Mitteilungen der Sternwarte der Ungarischen Akademie der Wissenschaften. Nr. 45: Pp. 10. Budapest: Sternwarte der Ungarischen Akademie der Wissenschaften, 1960). On the night of September 13, which was exceptionally clear in Hungary, an expanding dark spot was observed on the moon, near the crater Autolycus, by several astronomers at two independent observatories, Szabadsághegy and Baja, between the times 21h. 02m. 30s. U.T. and 21h. 07m., when it became too diffuse to observe. Traces of a similar phenomenon were found on photographs taken at the same time at Uppsala, Sweden. The co-ordinates of the dark spot (+ 0·03, + 0 45) agree well with those given for the impact point of *Lunik* 2.

Seventeenth-Century Bronze Finds in Eastern Nigeria

An interim report of excavations undertaken at Igbo-Ukwu, Eastern Nigeria, by Thurstan Shaw, appears in the November issue of *Man*. About twenty years ago some interesting finds, including bronze vessels, were unearthed by an African called Isaiah Anozie when digging out an underground cistern. Some of these objects are now in the museums at Jos and Lagos, and the facts of the find were duly reported in *Man*. Thurstan Shaw has now undertaken systematic investigations at the site and many more objects have been recovered from this and another nearby compound. They include pots, bowls, a swordhilt and scabbard, ornaments, rings, and a coiled snake on a spike, all in bronze. Of the two sites the first seems to have been a vessel or regalia store-place, and the second a burial. It is suggested that they were both connected with the former *Eze Nri*—the priest King of Umueri, who brought from the region of Idah an intrusive culture into Iboland. A seventeenth-century date and African workmanship are here tentatively postulated.

Indians of the Western Prairies

The Department of Northern Affairs and National Resources, Canada, has recently issued a bulletin in its Anthropological Series entitled *Indian Days on the Western Prairies*, which has been prepared by Marius Barbeau (Bulletin No. 163. Anthropological Series, No. 46. Pp. vi+234 and 165 plates. Ottawa: Queen's Printer, 1960. 4 dollars). The Bulletin is full of Indian tales and is well illustrated, sometimes in colour. But the work, of course, is a serious contribution to the study of the old inhabitants of the western prairies. There is a long introduction, where a general account of the tribes and their problems is given and the difficulties facing them are discussed. Most of the volume, however, is devoted to stories told by the Indians themselves, which give a vivid idea of the outlook on life of these people at the time of the coming of the white man, and of how they reacted to his arrival. There are a great number of illustrations of scenes and designs, mostly taken from paintings on skins. There are also portraits drawn in characteristic style by Langdon Kihn. The volume is of great value to anyone wishing to learn something of the civilization of these interesting peoples.

Diagnosis of Yaws

As increasing progress is made in the eradication of yaws in many countries, it becomes increasingly important to differentiate as clearly as possible between skin and other lesions not caused by yaws and those yaws lesions which they may closely resemble. At all stages of a mass campaign for the eradication of yaws, the clinical diagnosis should be as accurate as circumstances permit. However, in view of the limited time available in the initial treatment surveys and of the simple training given to many of the field staff, a certain proportion of diagnoses—probably about 10 per cent—may be expected to be erroneous. Such errors are all the easier to make as the skin has a limited number of reaction patterns, so that similar lesions may result from different causes. In the later stages of an eradication campaign, the manifestations of yaws have largely disappeared as a result of mass treatment with penicillin. It is then that many lesions not due to yaws become prominent, and at this stage accuracy of diagnosis assumes particular importance. The lesions of yaws were fully illustrated in C. J. Hackett's *An International Nomenclature of Yaws Lesions*, published by the World Health Organization in 1957. A new monograph, written by the same author in collaboration with Dr. L. J. A. Loewenthal, is intended as a companion volume to the earlier publication, and was prepared in response to requests for an illustrated handbook which would assist field workers in making accurate diagnoses (World Health Organization. Monograph Series No. 45: *Differential Diagnosis of Yaws*. Pp. 88 (52 figures). (Geneva: World Health Organization; London: H.M. Stationery Office, 1960.) 10 Swiss francs; 17*s*. 6*d*.; 3.25 dollars.

Hearing in Cetaceans

The skull of the Cetacea differs widely from the usual mammalian pattern; the relative sizes and positions of the bones forming it are much modified, and in many species they show a high degree of asymmetry. A complicated system of air sinuses connected with the Eustachian tube is associated with extensive venous plexuses in the basicranial region, the tympano-periotic bones are dissociated from the skull, and the bones of the middle ear with the tympanic membrane are also highly modified. Such peculiar conditions have long attracted the attention of comparative anatomists, whose observations are scattered through the literature of cetology. A recent paper (Fraser, F. C., and Purves, P. E., "Hearing in Cetaceans", *Bull. Brit. Mus.* (*Nat. Hist.*), 7, No. 1; 1960) reviews the history of the subject, and gives detailed comparative descriptions of the anatomy of the cetacean head based on original dissections, injected preparations, and radiographs. The authors had access to a large amount of unique material and have brilliantly illuminated much that was previously obscure. They have not, however, confined themselves to descriptive anatomy, for they have devised and carried out numerous elegant experiments to ascertain the functions of the various anatomical structures in the physiology of hearing. They have been able to show that the generally accepted theory which claims the Cetacea hear by bone conduction is not only erroneous but also impossible, and that in all the cetaceans the sense of hearing is the most important of the special senses. These points and many others are lucidly described and discussed in this scholarly and well-written paper, which is illustrated with numerous excellent plates. It is by far the most important work on the anatomy and physiology of the Cetacea to appear since Scholander's classic paper of 1940.

Effects of Maleic Hydrazide on Rust Fungi

Some interesting differential effects of maleic hydrazide on the growth of leaf and stem rusts of wheat are reported by D. J. Samborski *et al.* (*Canad. J. Bot.*, 38, 1; 1960). The effect of this substance was investigated, using intact plants supplied with maleic hydrazide through the roots, detached leaves floated on solutions of maleic hydrazide, and spores germinated on maleic hydrazide solutions in agar. In each instance the growth of leaf rust was inhibited at concentrations of maleic hydrazide which had no effect on the growth of stem rust. Some resistant wheat varieties became susceptible to rust after treatments with levels of maleic hydrazide which were not inhibitory to rust development. In some experiments, leaf rust races reacted differentially to maleic hydrazide. The authors consider that both nutritional and inhibitory factors may be involved in resistance and host–pathogen specificity.

Tenth Pacific Science Congress

THE tenth Pacific Science Congress is to be held at the University of Hawaii, Honolulu, during August 21–September 6, 1961. The Congress will be divided into nine Sections: Agricultural Sciences; Anthropology and Social Sciences; Biological Sciences; Conservation; Forestry; Geography; Geophysical Sciences; Public Health and Medical Sciences; Scientific Information. Each Section will include a number of divisions. Three special symposia are being organized: "Man's Place in the Island Ecosystem"; "Science Museums in the Pacific Area"; "Galapagos Islands: a Unique Area for Scientific Investigations". Further information can be obtained from the secretary-general, Mr. Harold J. Coolidge, Tenth Pacific Science Congress, Bishop Museum, Honolulu 17, Hawaii.

University News: London

APPOINTMENTS are announced as follows: Dr. D. R. Cox, reader in statistics at Birkbeck College, to the University chair of statistics tenable at that College; Dr. E. F. Scowen, reader in medicine at St. Bartholomew's Hospital Medical College, to the University chair of medicine tenable at that College; Mr. Arthur Summerfield, lecturer in psychology at University College, to the chair of psychology tenable at Birkbeck College; Dr. Donald Rogers, senior lecturer in physics at University College, Cardiff, to the University readership in chemical crystallography tenable at the Imperial College of Science and Technology from October 1. Titles within the University of London have been conferred as follows: professor of mathematics, on Dr. K. F. Roth, in respect of his post at University College; reader in medicine, on Mr. P. H. Sanderson, in respect of his post at St. Mary's Hospital Medical School; professor emeritus of biochemistry, on Prof. W. Robson, on his retirement from the professorship of biochemistry at King's College; reader in mathematics, on Dr. F. A. E. Pirani, in respect of his post at King's College; reader in mathematics, on Dr. S. Raimes, in respect of his post at the Imperial College of Science and Technology. Dr. F. A. Goldsworthy was appointed to the readership in applied mathematics at Queen Mary College (see *Nature*, 189, 22; 1961), but was unable to accept the appointment.

Announcements

THE following awards have been made by the president and council of the Royal Astronomical Society: *Gold Medal*: Prof. Herman Zanstra, until recently professor of astronomy in the University of Amsterdam, for his work on gaseous nebulæ; *Eddington Medal*: Prof. Hans A. Bethe, professor of nuclear physics at Cornell University, for his outstanding identification of energy-generating processes in stars.

MR. J. A. CHALDECOTT, deputy keeper in the Department of Physics, Science Museum, South Kensington, has been appointed keeper of the Science Library in succession to the late Mr. H. T. Pledge. Mr. G. W. B. Lacey, assistant keeper, has been appointed deputy keeper in the Department of Aeronautics.

MR. M. A. L. BANKS, a managing director of the British Petroleum Co., Ltd., has been appointed a member of the Advisory Council on Research and Development to the Ministry of Power. The Council, which advises the Minister on the scientific aspects of his statutory duties, was recently reconstituted and its terms of reference amended so as to emphasize that its advisory functions cover all aspects of research and development concerned with fuel and power from the laboratory to industrial application.

THE Industrial Science Achievement Award of the American Association for the Advancement of Science has been awarded to the Bell Telephone Laboratories, Inc., for its achievements in the field of universal communication during the year 1960. Among the work mentioned was: long-distance communication by reflecting radio-signals from *Echo* 1, the world's first electronic telephone switching system, the optical maser and solid state materials.

APPLICATIONS are invited by St. John's College, Cambridge, for the Kenneth Craik Research Award, which is available for the assistance of persons engaged in postgraduate research, preferably in physiological psychology. The value of the Award will be £450 a year. Applications should be sent to the Master, St. John's College, Cambridge, so as to reach him not later than April 1, 1961.

A NEW colour magazine, *Knowledge*, has appeared. It is intended, by an easy appeal, to give children a glimpse of "the strange variety of things that the world has to show", as the editorial states. There is a South American edition too, and similar editions will be forthcoming from other countries; a cultural exchange of articles is planned. The magazine costs 2*s*. and appears every Monday (Purnell and Sons, Ltd., 37 Hertford Street, London, W.1).

THE Royal Society has available for loan to film societies, educational institutions, schools, etc., a 16-mm. sound film in colour which runs for about 40 min. entitled "South from Chiloé" at a nominal service charge of two guineas per showing. This film tells the story of the Society's expedition to Southern Chile during the period October 1958–February 1959 which went to study the flora and fauna of this little-known region of the world. Requests for hire should be addressed to the Assistant Secretary, Royal Society, Burlington House, Piccadilly, London, W.1.

THE Society of Leather Trades' Chemists is organizing a symposium on "The Technology of Drying Fibrous Materials with Particular Reference to Leather" during March 23–24. The symposium will be held in the Physical Chemistry Department, University of Cambridge. Further information can be obtained from G. H. W. Humphreys, 3 The Grove, Radlett, Herts.

A SYMPOSIUM on "Microchemical Techniques" is being organized by the Metropolitan Microchemical Society under the sponsorship of the International Union of Pure and Applied Chemistry. The symposium will be held at the Pennsylvania State University during August 13–18. Further information can be obtained from Mr. Howard J. Francis, jun., c/o Pennsalt Chemicals Corporation, P.O. 4388, Philadelphia 18, Pennsylvania.

NEW RESEARCH LABORATORIES OF HILGER AND WATTS, LTD.

SINCE the Second World War, the idea conjured up by the expression 'scientific instrument' has changed. It is well known that electronics has invaded every field of measurement, including the optical field, but it is less realized that instruments have tended to become larger. They have done so not only because of the addition of electronic units but also because this in turn has made possible complexities that would previously not have been considered.

To take one example, the quality of steel during the War was controlled by using the large quartz spectrograph, the photographic plates exposed in which carried the spectra of some twenty samples together with calibration spectra. The blackness of the lines at the appropriate places was measured with a microphotometer, reading from a galvanometer scale. Nowadays, when a charge of 70 tons of steel may be prepared and poured in 12 min., the foreman must have the analysis of a sample in as few minutes as possible if he is to have time to take any necessary action to rectify the charge. The automatic spectrograph or 'polychromator' takes up three or four times the space of the equipment previously used, but it provides an analysis of as many as thirty elements in about 3 min. in a typical case. To determine the carbon, phosphorus and sulphur in a sample, instruments are made that use the vacuum ultra-violet region of the spectrum as well as the air-penetrating ultra-violet region.

This trend, as well as the rise in numbers of instruments required, has induced Messrs. Hilger and Watts, Ltd., to build a new research laboratory at Camden Town, London, N.W.1, as an extension of their headquarters building there. Since the comprehensive damage to the factory caused by a flying bomb in 1944, research on instruments for analytical and kindred purposes has been carried out in rooms spread over the factory and adjacent outbuildings. (This does not apply to the research on surveying, photogrammetric and engineers' measuring instruments being done at the firm's Camberwell factory.)

The use of the new building was inaugurated by Sir George Thomson on December 15. The ceremony took the form of visiting the laboratories, in which normal work was in progress, and a luncheon attended by some 150 guests. The guests were drawn from among university physical and chemical departments, medical and government laboratories, and the research laboratories of industry.

Sir George was introduced by the chairman of the Company, Mr. G. A. Whipple, who is the present master of the Company of Scientific Instrument Makers. Mr. Whipple referred to the growth of the firm, which now has more than 2,000 employees, an associated company in the United States, and branch offices in Canada and Western Germany. He thanked the architect (Mr. J. Francis Smith), the builders (Messrs. Mullen and Lumsden), and the quantity surveyor (Mr. D. T. C. Thompson).

Sir George then proposed the toast of the Company and the new laboratories. He spoke of the 'pure' research worker's debt to industry, in that the needs of industry for large and complex instruments, which had to be reliable, encouraged the scientific instrument firms, which in turn could provide better instruments for pure research. Hilger and Watts was such a firm: "Twyman proved to industry the value of spectroscopic analysis. The demand so created made expansion possible, and its latest stage we are inaugurating to-day. From this, we benefit by the increased scale of the firm and by the great improvement this has made possible in design, which has given this great firm the enormous prestige it now holds throughout the world and which we are happy to be here to-day to celebrate".

Dr. A. C. Menzies, the firm's director of research, said in his reply that he had been concerned in his

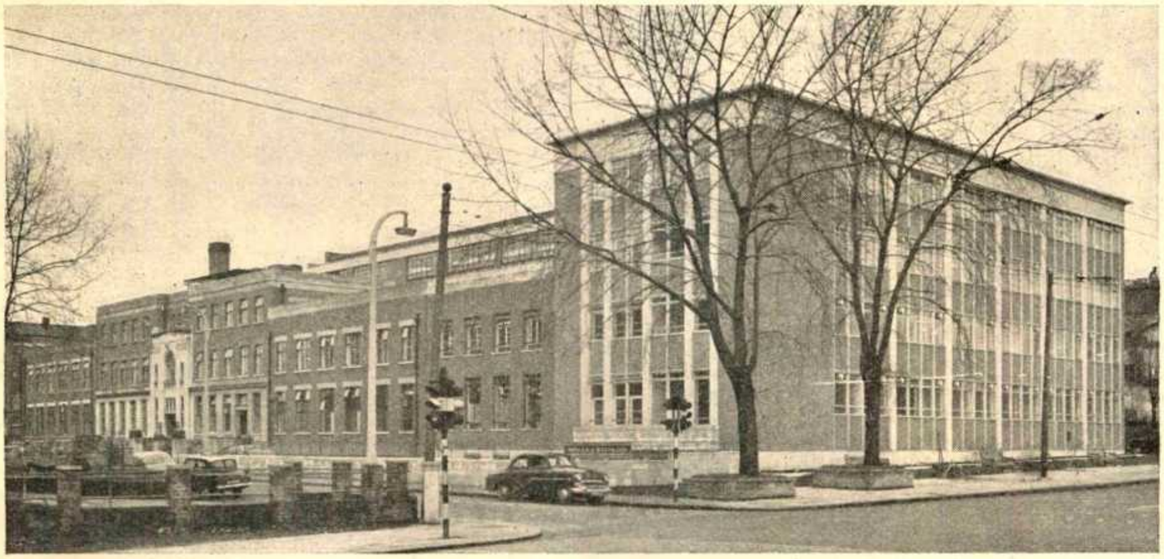

Fig. 1. New Research Laboratory of Hilger and Watts, Ltd.

life with the building of three laboratories. However, it was only with the third of them, this new laboratory at Camden Town, that he had had the pleasure of contributing little but the expression of his requirements. This was the result of having good colleagues. He had worked with Sir George Thomson during the War, and it was at Sir George's instance that he had come to Hilger and Watts to succeed Mr. Frank Twyman. Sir George had added with a smile that the job was an easy one, so Dr. Menzies had frequently overcome difficulties since then by reminding himself that they could not exist. When he started with the firm, he turned one of the smallest rooms in an outside building into a dark room; "it had the advantage that water was laid on already". Such conditions made him resolve to work for a satisfactory, attractive place where his colleagues could work comfortably and effectively. Now they had it.

After the luncheon, the guests toured the laboratories and saw research being undertaken into spectroscopic analysis by emission, X-ray diffraction and X-ray fluorescence; infra-red absorption; optical design; absorption spectroscopy; optical instruments for the control of chemical processes; electron-spin resonance spectrometry; and generators for arcs and sparks. N. J. GOODMAN

SCIENTIFIC RESEARCH IN AUSTRALIA

THE twelfth annual report of the Commonwealth (of Australia) Scientific and Industrial Research Organization, covering the year ended June 30, 1960, departs considerably from the form of previous years*. To facilitate its presentation to Parliament early in the 1960 Budget sitting, the chapters describing detailed technical matters characterizing previous reports have been replaced by a brief account of the more important developments during the year, and the details are to be published separately as a research review. The bulk of the present report consists of lists of published papers, and the membership of the Advisory Council, State Committees and staff and finance.

Of the expenditure of £9,489,741, £8,862,160 was on normal research activities and £144,600 on grants to outside bodies. Among new arrangements with the universities are noted support of an investigation by the Department of Zoology, University of Melbourne, into the effects of chromosomal inversion on viability of grasshoppers; collaboration with the University of New South Wales in an investigation of tritium-labelling techniques; continuation of research at the Australian National University into aggregation and denaturation of protein; and an extended loan to the Electrical Engineering Department, University of Tasmania, of the differential analyser developed by the Mathematical Instruments Section. Construction of the giant radiotelescope at Parkes, New South Wales, for the Division of Radiophysics is expected to be complete early in 1961.

Among the more important developments of the year noted in the chapter "Progress in Research" are the solution of serious problems preventing the development by irrigation of large areas of potentially fertile but intractable soils in the Riverina by using low concentrations of gypsum in the irrigation water to make the surface clays permeable. A study of the characteristics of coats of cattle in the tropics showed that gain in weight can be predicted much more accurately from the type of coat than from actual records of past gain in weight. A new process by which cheese is matured in a plastic wrapping has been adapted for Australian conditions, and a new kind of anti-fungal compound, 'Pisatin', has been isolated from peas. A strain of cocksfoot grass, introduced from Brignolles in France, has proved outstandingly productive as a component of winter pastures near Canberra and has persisted well under heavy stocking. Up to 90 per cent control of rabbits has been achieved with sodium fluoroacetate using a suitable bait; but its effective use depends on intelligent application of accurate knowledge of feeding habits and behaviour of the rabbit. Striking increases in beef production have been obtained in grazing trials with a grass-legume mixture in native spear grass country near Gladstone, and further evidence has been obtained of the primary importance of nutritional factors in determining the mortality among new-born lambs. The compounds causing certain off-flavours in butter have been identified, and most of the conditions under which these flavours develop have been recognized. Research at Crookwell has shown that sulphur is an essential constituent of the soil organic matter, and that lack of sulphur often limits the rate of build-up of soil fertility under improved pastures.

The first commercial plant for the manufacture of fully mechanized Cheddar cheese has been installed and successfully operated, and a field experiment in North Queensland suggests that control of the cattle tick can be greatly improved both by pasture spelling and strategic dipping. Equipment has been designed to overcome the difficulty of measuring the temperature of biscuits in a long conveyor-type oven due to the very limited access to the interior of the oven, and the method for producing washable non-iron effects in wool fabrics has reached commercial application. Two new statistical techniques have been developed for accurate spatial prediction of rainfall.

Polyethylene box liners have been used to increase significantly the storage life of four main varieties of apples. A new pre-steaming procedure has been developed which is greatly improving the drying of refractory timbers with a saving of 20 per cent in the time of drying with some species. It has now been found that sugar-cane wax as it occurs on the cane consists chiefly of polymerized long-chain aldehydes, with lesser amounts of long-chain alcohols. A study has also been made of the waxes coating the tubercule and diphtheria bacteria.

Investigations of the hardening of brass have proved that alpha brass is 'ordered' after slow-cooling treatments, and that the degree of order can be considerably increased by isothermal annealing at low temperatures. X-ray methods have been further developed for the determination of the structures of large molecules, by applying a high-power X-ray generator and by collecting intensity data at low temperatures. In extensive tests at Umberumberka (Broken Hill) and at Lake Corella reductions of as much as 50 per cent of the normal evaporation have been obtained in still conditions by dusting with solid hexadecanol, but when the wind velocity exceeds 5 m.p.h. the saving is very much less. The first model of the 'Evaporotron', an instrument designed to yield direct readings of evaporation from natural sources on the eddy-flux principle, has been completed. Fundamental study of the physics of wool has established the existence of a transition temperature above which irreversible change occurs if the wool fibre is strained, and also that fibres of animal tendon have a transition temperature only slightly above normal blood temperature. Investigations of the retarding of non-enzymic browning of stored food by addition of sulphur dioxide or bisulphite indicate that the bisulphites may act through oxidation of the carbonyl compounds found in the aldose-amine reactions preceding browning.

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* Commonwealth of Australia. Twelfth Annual Report of the Commonwealth Scientific and Industrial Research Organization for the year ending 30th June, 1960. Pp. ii + 129. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1960.)

STRONTIUM-90 IN HUMAN DIET IN THE UNITED KINGDOM, 1959

A REPORT by the Agricultural Research Council contains the results for the second year of the survey of strontium-90 in human diet in the United Kingdom which was initiated by its Radiobiological Laboratory in 1958*. Two types of investigation are described: a country-wide survey, which enables the mean ratio of strontium-90 to calcium in the average diet of the population to be assessed, and local studies in areas where the quantities of strontium-90 in the diet are likely to exceed the average.

The general organization of the country-wide survey was similar to that in 1958, though some additional foods were examined. The major effort was devoted to milk; bulk samples were prepared for analysis from milk collected twice each month from some 200 depots which handled more than 40 per cent of Britain's total production. Potatoes, leaf vegetables, flour, cheese, eggs, proprietary cereal-based infant foods and tea were also examined. It has already been reported that the quantity of strontium-90 in milk rose appreciably in the early part of 1959 with the result that the mean value for the twelve months ending in the middle of the year was 40 per cent greater than it had been six months earlier. Afterwards, however, the level of strontium-90 in milk decreased and, in the latter part of the year, it was similar to those observed in 1958; thus, the mean value for the preceding twelve months changed little between June and December. The country-wide mean values at the end of June and December 1959 were 11·7 and 12·2 μμc./l. (9·6 and 9·8 μμc./gm. calcium), respectively.

It is estimated that the mean ratio of strontium-90 to calcium in the average diet of the whole population in the United Kingdom during 1959 was 9·0 μμc./gm. and that it did not exceed 15 μμc./gm. in the diet of any large section of the population. Because of the relatively high levels of contamination early in the year, these values are about 50 per cent higher than those estimated for 1958. The estimated level in the diet of the population as a whole, however, represents less than one-twentieth of the maximum permissible level recommended by the Medical Research Council.

During the year, investigations were intensified to identify localized areas where the combination of high rainfall and agricultural factors could lead to abnormally high levels of strontium-90 in human food. Thirteen individual farms or groups of small adjacent farms were examined in wet upland areas in England, Wales, Scotland and Northern Ireland. All these areas are small, and only a fraction of the food consumed in them is produced locally. The results of these studies, however, enable conclusions to be reached as to the hypothetical maximum levels of strontium-90 which could occur in the diet of any individual. If it is assumed that an individual could consume the most highly contaminated foods of different types which have been found in these investigations (and these were, in fact, produced in widely separated parts of the country), the ratio of strontium-90 to calcium in the total diet would be 80 μμc./gm. This value, which is one-tenth of the maximum permissible level recommended by the Medical Research Council for individuals, is in fact considerably in excess of the likely levels in the diet of any one person. Moreover, it is less than one-third of the maximum permissible level for the whole population.

Milk continues to be the component of diet which contributes the largest quantities of strontium-90. The ratio of strontium-90 to calcium in milk is, however, similar to, or less than, that in many other foods. Since the concentration of strontium-90 in newly formed human bone depends on the ratio of strontium-90 to calcium, rather than on the absolute quantity of strontium-90 in the total diet, milk should not be regarded as giving rise to any special hazard.

Variations in the composition of diet usually have only small effects on the mean ratio of strontium-90 to calcium in the total daily intake. The only substitution in a normal diet which is likely to have a significant effect is that of wholemeal bread for white bread. The calculations in this report for the average mixed diet are for that containing white bread. The substitution of wholemeal bread which contains a higher quantity of strontium-90, and does not generally contain added calcium of mineral origin, would not, however, increase the ratio of strontium-90 to calcium in the average mixed diet by more than 6 μμc./gm. This increase is less than one-fortieth of the maximum permissible level for the general population.

Because climatic factors can exert considerable effects on the quantities of strontium-90 and of calcium which are absorbed by plants, and hence on the levels in human diet, it is not possible to make quantitative predictions as to the situation in the immediate future. There is, however, considerable evidence that strontium-90 which has hitherto been released into the atmosphere has already exerted its major effect; decreasing levels in human diet may therefore be expected in the future.

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* Agricultural Research Council. Radiobiology Laboratory. Report No. 3.: Strontium-90 in Human Diet in the United Kingdom, 1959. Pp. x+58. (London: H.M. Stationery Office, 1960.) 3s. 6d.

RESEARCH IN PARASITOLOGY

THE autumn meeting of the Parasitology Group of the Institute of Biology was held in the Zoology Department of Birkbeck College, London, on October 28. The meeting was very well attended and was devoted to five papers under the general title "Recent Advances in Fields of Parasitological Research". A short discussion followed each paper.

During the morning session the chair was taken by Dr. Ann Bishop. The first paper, given by Mr. Bernard Weitz (Lister Institute, Elstree), was on "Trypanosomiasis". Mr. Weitz pointed out that eradication of the disease depends on the control of the vector or the elimination of the reservoir hosts. *Glossina* can be controlled by bush clearing and, more recently, by the critical use of insecticides. Eradication of the insect by the sterilization of males by γ-irradiation is not practicable at present. Destruction of game removes the source of food of the vector and the parasite reservoir simultaneously. Consideration of the feeding habits of *Glossina* might permit the partial destruction of game, resulting in starvation of the fly. Animals which are frequently bitten by *Glossina* are usually tolerant to artificial infection, and, conversely, those which are infrequently fed on by the insect develop a parasitæmia and die more rapidly. On the other hand, it is difficult to understand how animals which are seldom or never bitten by tsetse become frequently infected unless by some other method of transmission. It is not clear whence the infection in the fly is derived. Immunity of animals can be assessed by a number of tests, but the specificity or sensitivity of the available serological techniques has led to confusing conclusions. The respiration test was recently used to measure the immune response of apparently resistant breeds of cattle. Recent attempts to investigate the antigenic pattern of trypanosomes showed that soluble antigens, released in the bloodstream of infected laboratory animals, influence infection. Laboratory animals immunized with these soluble antigens are protected against infection. Other antigens are released on mechanical disintegration of the trypanosomes. The occurrence of these antigens has been confirmed in the natural disease. Drugs are an important means of control of the disease but cannot be considered as a permanent measure. The local eradication of the disease must depend on concerted action by the use of all available methods of control.

This was followed by a paper by Dr. B. A. Newton (Department of Biochemistry, Cambridge) on "The Mode of Action of Quaternary Ammonium Trypanocides". The early work of Lock (1950) and Ormerod (1951, 1952) on the mode of action of ethidium bromide and 'Antrycide' indicated that these compounds differed markedly from the older type of trypanocidal drug (for example, trivalent phenyl arsenoxides, stilbamidine and acriflavine). Cytochemical studies led Ormerod to suggest that both compounds act in a similar manner by combining with, and inactivating, cytoplasmic ribonucleoprotein. Stimulated by these observations, work has been carried out on the action of ethidium bromide and 'Antrycide' on the trypanosomid flagellate, *Strigomonas oncopelti*, growing in a chemically defined medium. It has been found that these two drugs differ in their effect on the growth of *Strigomonas*; ethidium inhibits growth after about one generation, whereas 'Antrycide' changes the pattern of growth from a logarithmic form to a linear form. Examination of drug-treated cells has revealed an abnormal ratio of ribonucleic acid to deoxyribonucleic acid following ethidium treatment; no such change was observed after 'Antrycide' treatment. A more detailed investigation of the effects of these two compounds has shown that ethidium rapidly inhibits the synthesis of deoxyribonucleic acid, but permits the synthesis of ribonucleic acid and protein to continue for a limited time; antrycide, on the other hand, specifically inhibits the incorporation of purines into nucleic acid, but does not inhibit the net synthesis of nucleic acid so long as the organisms are able to synthesize purines. This action of 'Antrycide' is associated with the pyrimidine portion of the molecule. Examination of an 'Antrycide'-resistant strain of *S. oncopelti*, which has been developed *in vitro*, suggests that resistance is not associated with a change in cell permeability, or with a change in the affinity of cytoplasmic nucleoprotein for the drug. The possible nature of resistance was briefly discussed.

The afternoon session, under the chairmanship of Dr. E. L. Taylor, opened with a paper by Dr. C. Ellenby (Department of Zoology, King's College, Newcastle) on "Plant Nematology". Dr. Ellenby confined his remarks almost entirely to the cyst-forming eelworms of the genera *Heterodera* and *Meloidogyne* and considered aspects related to secretions, not only from the plants but also from the nematodes themselves, and their influence on nematodes and plants respectively. Among the points mentioned were the secretions from bacteria and various host and non-host plants, the suppressant effect on eelworm populations of diffusates from the roots of marigold (*Tagetes*), hatching factors (including his own work and that of Schreiber), and the influence of nematodes on plant growth, trap formation in fungi and gall formation in the host plants. Gall formation and specificity were then outlined and this led to a consideration of 'resistance'. Finally, sex ratio and sex determination were discussed in the light of recent work on the possibility of sex reversal in a species of *Meloidogyne*.

The second paper was given by Dr. C. A. Wright (British Museum, Natural History) under the title "Malacology". Development of recent work on the identity of intermediate hosts of trematodes of medical and veterinary importance has shown the existence of a greater degree of host restriction at the molluscan stage of the life-cyle than was formerly accepted. This has led to the idea of a parallel evolution between trematode species and their molluscan hosts, and has emphasized the importance of infra-specific taxonomy, often at the population-level, in the study of the hosts. Originally the taxonomy of molluscs was based on shell characters alone, characters which are now known to be very variable, and the result was the naming of large numbers of redundant 'species'. Later workers used the radulæ of gastropods as a further aid to identification, but, here again, insufficient attention was given to individual variations. The study of anatomical characters, particularly of the genital system, has been developed relatively recently and, in many cases, has provided useful taxonomic information. However, the plasticity of muscular structures and the effects on them of different methods of fixation, and also differences due to the maturity of the animal, necessitate a very careful interpretation of these anatomical data. Work is at present directed towards attempts to supplement the morphological criteria for species discrimination by biochemical characters, and the application of techniques such as paper chromatography of mucus, electrophoresis of blood proteins and systematic serology based on tissue extracts is beginning to provide a sounder basis for the systematics of the molluscan intermediate hosts of trematodes.

The last paper of the meeting was by Dr. C. Horton-Smith (Houghton Poultry Research Station) and was on "Poultry Parasites and their Control". A short review of the Coccidia of poultry introduced the main theme of the paper which was a discussion of the chemotherapy of coccidiosis, and the problems of developing drug resistance in *Eimeria tenella*; this occurs when the organism is exposed, over extended periods, to low and therapeutic concentrations of anti-coccidial compounds. The reactions of different strains of the same species, as well as the differences in species response to the same drug, were considered. The mode of action of anti-coccidial drugs, including the potentiation of sulphonamides by pyrimethamine, and the effect of anti-coccidials on developing immunity were also discussed. The selective action of certain sulphonamides on schizogony and, in particular, the second generation, associates this stage with the major role in the stimulation of resistance to *E. tenella*. This is amplified by the results of recent work. Finally, the mechanism of response to *E. tenella* was considered. Precipitating antibodies have been demonstrated as a result of the oral administration of oocysts, but the significance of these precipitins is, as yet, unknown.

The proceedings of this meeting will not be published in full. SHEILA WILLMOTT

NATURAL RUBBER RESEARCH

THE Natural Rubber Research Conference, held in Kuala Lumpur during September 26–October 1, was sponsored by the Rubber Research Institute of Malaya and the Government of the Federation of Malaya. More than 280 scientists from 24 countries and all five continents attended, and considered some 76 research papers, making this by far the largest gathering ever to discuss research on the production and utilization of natural rubber.

As in the case of other industries based on natural products, the range of research sponsored by the natural rubber industry is exceedingly wide, and the papers were representative of many scientific disciplines. Thus, the topics discussed ranged from the role of trace elements in the nutrition of the *Hevea* tree, to biochemical and biophysical investigations of latex, and to the design of rubber components in structural engineering.

During the week, the conference heard lectures from three distinguished guests: Prof. G. E. Blackman, Prof. James Bonner and Prof. G. Gee.

Biological Studies of the Rubber Tree

Prof. Blackman spoke on "The Stimulation of Latex-Flow by Plant-growth Regulators". In tracing the history of the development of successful yield stimulant mixtures, which increase the flow of latex after tapping, he showed how this had been dependent on fundamental research into the activities of plant-growth substances. It had been estimated that several million pounds worth of additional rubber had been produced in Malaya during the past four years by the use of these stimulants. Prof. Blackman made it clear that there was still much to be learnt about the exact mechanism of the yield stimulation process; but, as other papers presented to the conference indicated, this is the subject of active work. Other authors discussed such matters as the formulation of stimulant mixtures, their application to the tree and factors influencing the response obtained. A new aspect of yield stimulation was provided by a report that yield increases may also be obtained by the use of antibiotic treatments.

Of no less importance than yield stimulation is the development of intensive tapping methods which permit the maximum exploitation of older trees. Experiments are now in progress to determine the optimum combinations of tapping systems and yield stimulation techniques to give the highest possible yield throughout the life of the rubber tree.

Several papers considered various aspects of the breeding and selection work which has always found a prominent place in the programmes of rubber research organizations. The newer varieties now being planted are capable of giving at least four times the yield of the unselected material used in the early years of the century. It is believed, however, that all the high-yielding Eastern varieties are susceptible to the South American leaf blight disease caused by the fungus *Dothidella ulei*. A number of speakers directed attention to the potential threat of this disease to the rubber-growing areas of Africa and Asia, and called for closer international co-operation in the development of high-yielding resistant varieties.

In the absence of any suitable method of true vegetative propagation, *Hevea* clones are still multiplied by bud-grafting on to seedling rootstocks. A procedure was described for bud-grafting seedlings considerably younger than those used at present, and an account was given of the successful rooting, under a mist spray, of leafy cuttings of a number of important *Hevea* clones. The commercial value of these new techniques remains to be evaluated, but the rooted cuttings are already providing an important source of uniform material for experiments, such as those on stock–scion effects. Meanwhile, techniques of growth analysis have been developed to give a clearer picture of the effects of rootstocks on scion growth and the variation in the pattern of growth in different clones.

In rubber, as in other crops, the standard method of deciding fertilizer requirements has always been by long-term field experiments. It now appears that leaf and latex analysis may offer an important complementary method. This work, pioneered in Viet-nam, is now being vigorously pursued elsewhere.

As deficiencies in the major elements are corrected, more attention is being paid to the minor ones. It was emphasized that the applications of nitrogen, phosphorus and potassium so often used are inadequate on the generally poor soils of the humid tropics. Magnesium is as important as potassium, and deficiencies in several other elements are known to occur. These factors may greatly affect, and even invalidate, measurement of response to the three main elements.

The tree is now, more than ever, regarded as a part of a complex ecological system, and several papers described the marked effects of different cover species on tree growth. The beneficial effects of creeping legumes were particularly emphasized. Despite the large volume of present work on chemical weed control, there was only a single paper on this subject.

In the sessions on pests and diseases, interest centred on the root diseases, which can cause very serious losses to the rubber grower. One of the difficulties of the plant pathologist in this field was illustrated by a paper which showed that the usually accepted name for the pathogen of the common white root disease of *Hevea*, *Fomes lignosus*, was open to grave objection on taxonomic grounds. The potential hazards of the invasion of the cut surfaces of stumps by wind-borne spores, at the time of replanting, were emphasized, and it was shown that simple and inexpensive procedures could greatly reduce the toll of root disease in rubber plantings. Closely related to this problem is the study of factors affecting the rate of decay of rubber stumps and the role of biological agencies in the removal of this potential source of disease. The possible role of fungicides in the control of root disease was also discussed.

Fundamental work on the raw material of the natural rubber industry, *Hevea* latex, was well represented at the conference. Latex is very far from being a simple suspension of rubber particles in a watery medium. It contains a bewildering array of other particles which are under active investigation, in which use is made of the electron microscope, advanced forms of light microscopy and the ultracentrifuge. Workers in this field now appear to be agreed in regarding latex as a cytoplasmic fluid, and, as these studies develop, it will be interesting to see whether the particles which accompany the hydrocarbon in latex vessels have exact analogues among the known components of other plant cells, or whether they represent hitherto unknown entities.

It is now established that the process of rubber formation occurs within this complex system and it may be made to continue, experimentally, even after the latex has left the tree. An elegant account was presented of the way in which the acetate molecule is converted to rubber hydrocarbon in *Hevea* latex. Members of the conference also heard Prof. Bonner give a striking review of the whole process of rubber biosynthesis and set out suggestions for future research on the unsolved problems of the subject.

The biological complexity of latex is, of course, accompanied by a corresponding chemical diversity. The conference reviewed recent progress in the study of the many substances which accompany the rubber hydrocarbon. As with other fields of biological chemistry, these studies on the non-rubber constituents of latex have gathered momentum in recent years, with the development of new methods of chromatography and electrophoresis.

The biological character and diverse composition of natural latex render it highly susceptible to bacterial attack, and much time and effort have to be devoted to preventing the adverse technological effects which result. The subject is a complex one, and it gave rise to lively discussions during the conference. It appears to be agreed, however, that contamination of the latex begins within the cut ends of the latex vessels themselves and is reinforced at various stages in collection and processing. Measures to control it are already in widespread use and improvements are constantly being sought.

Chemistry and Technology of Rubber Preparation

Knowledge of the structure of the rubber molecule itself is still advancing, and new information is being obtained by the use of the electron microscope and light-scattering techniques. New data were also presented on the variety of reactions which the hydrocarbon can undergo. This reactivity has been turned to advantage to produce modified rubbers, such as the graft polymers. On the other hand, it also leads to the troublesome spontaneous change in properties known as storage hardening. The conference heard an account of a successful method for the inhibition of storage hardening based on a new theory that this change is due to the presence of aldehyde groups.

The bulk of the industry's revenue is still earned by sheet, crepe and other forms of dry rubber. Investigations were reported which should help the producer to avoid defects which down-grade his rubber when it is judged by the almost universal method of visual classification. There were also reports of work aimed at preparing dry rubber in novel forms which would be graded by technological properties rather than by appearance.

Concerning special dry rubbers, the conference heard of the increased interest in 'superior processing' rubbers, which have greatly improved extrusion characteristics. Several papers showed that new advantages and uses are being found and that, with this spur, new low-cost methods of imparting superior processing properties are being discovered. None of the rubbers produced by these alternative techniques possesses the all-round advantages of standard superior processing rubber, but they show promise for applications where the use of the standard product would be uneconomic.

Research and development are encouraging new applications of rubber in many fields. In the textile industry, natural rubber latex is now being used increasingly in the manufacture of non-woven fabrics and in carpet backings. New design methods are enabling engineers to employ rubber components with the same degree of confidence as those of the older structural materials. Further work is also giving road engineers a more detailed picture of the practical benefits to be gained by incorporating various types of rubber into bituminous road-binders.

Testing methods for quality and processability of latex concentrate were described, together with clonal influences on latex foam properties. On the subject of skim rubber, the by-product of the latex concentrate factory, the conference heard how processing methods could affect its properties. Methods for the treatment and utilization of factory effluents were also discussed.

Finally, in his address, 'New Methods of Elastomer Synthesis and their Impact on Natural Rubber", Prof. Gee brought into the open the question that had formed the background to many of the discussions during the week: "Can natural rubber, even when produced by the most economical means, meet the challenge of the new synthetics ?" Prof. Gee's answer was "Yes", provided the natural rubber industry continued and intensified its fundamental research and technical development in the right directions. He showed that no single elastomer is supreme in all respects and that each of them, including natural rubber, can hold its place in the expanding market. He also described the striking results which may be obtained by blending natural and synthetic rubbers and how the presence of the reactive groups influences this blending.

There can be little doubt as to the success of the conference in bringing current ideas on natural rubber research into sharper focus and in stimulating new approaches to the many problems raised. The proceedings of the conference are to be published early in 1961, and those wishing to obtain copies should write to the Director, Rubber Research Institute of Malaya, P.O. Box 150, Kuala Lumpur, Malaya.

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ELEVENTH GENERAL CONFERENCE OF WEIGHTS AND MEASURES

THE eleventh General Conference of Weights and Measures was held in Paris during October 11–20 under the chairmanship of M. Barrillon, president of the Academy of Sciences. Thirty-two States were represented at the Conference, which was held at 19 Avenue Kléber, in the former Hotel Majestic now taken over by the Ministère des Affaires Étrangères for use partly as offices and partly as a meeting place for national and international conferences. One epoch-making scientific decision was taken, namely, to re-define the metre in terms of a natural atomic standard, the wave-length of light, thus deposing the platinum–iridium metre bar—the International Prototype Metre—from the supremacy it has held in the field of length measurements since 1889. Three very important administrative and financial resolutions were passed which are crucial to the future development and efficiency of the weights and measures organization. Before dealing with these and other decisions and with an unfinished effort of the Conference to modernize the international treaty on which its authority is founded, it would be well to give a brief outline of the history and development of the whole organization.

International co-operation in scientific metrology depends on the Convention du Mètre, a treaty signed in Paris by the plenipotentiaries of seventeen sovereign States on May 20, 1875. An agreement was thereby made to establish and maintain, at joint expense, a central laboratory and office, known as the Bureau International des Poids et Mesures, with the primary duties of maintaining, improving and extending the use of the metric system. The treaty organization, which the United Kingdom joined in 1884, now represents thirty-eight States (Argentina, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, Czechoslovakia, Denmark, Dominican Republic, Finland, France, Germany, Hungary, India, Indonesia, Ireland, Italy, Japan, Korea, Mexico, Netherlands, Norway, Poland, Portugal, Rumania, Spain, Sweden, Switzerland, Thailand. Turkey, the United Kingdom, the United States, Uruguay, the U.S.S.R., Venezuela and Yugoslavia (Indonesia and Venezuela joined after October 1960). It acts under the authority of the General Conference of Weights and Measures, constituted by delegations nominated by the Governments of the member-States meeting every six years, with the aid of the International Committee of Weights and Measures—an elected executive body of eighteen members (each from a different State) meeting every two years. This Committee administers the scientific and financial affairs of the Bureau International in between general conferences, drafts recommendations relating to units and standards of measurement with the help now of six expert advisory committees and acts as a steering agency during General Conferences.

The Convention was revised on October 6, 1921, mainly to extend the work of the Bureau International des Poids et Mesures to the standards of electricity and photometry. Up to that date the Bureau International, as custodian of the International Prototypes of the Metre and Kilogramme, was chiefly concerned in its laboratory work with the maintenance, by means of periodical comparisons, of the various national copies of these prototypes issued to the member-States in 1889 and afterwards. The derived standards of volume (or capacity), density and atmospheric pressure naturally came within the responsibilities of the Bureau International ; and, as the length of a material standard is dependent on its temperature, a practical scale of temperature was maintained. As a consequence of the 1921 revision, high-class electrical and photometric equipment was installed at the Bureau International for periodic intercomparisons of groups of standard resistors, standard Weston cells and standard lamps sent in by the national laboratories. In this way, just as for the national metres and kilogrammes, the uniformity of the electrical and photometric standards maintained by the various countries is readily checked, and any individual divergences from the general average which are unusual are identified and reported.

During recent years the rapid development of applications of radioactivity and the ionizing radiations in the scientific, medical and technological fields has created an urgent need for a central standards laboratory to work on the basic metrology of ionizing radiations and to correlate the standards and measuring techniques developed in the national and other specialized laboratories. In 1958 the International Committee established its expert advisory committee for the ionizing radiations which has produced proposals for a new Bureau International des Poids et Mesures laboratory and equipment, together with a general plan of the work to be done and the staff required. Recommendations embodying these proposals were submitted to the eleventh General Conference by the International

Committee, with others for: (1) increasing the staff and modernizing the equipment in existing sections of the Bureau International; (2) changing the rule by which the total annual subvention for maintenance of the Bureau International is apportioned among the member-States. All these recommendations were unanimously adopted and the Conference thereby sanctioned:

(*a*) The creation of a new section at the Bureau International des Poids et Mesures for work on the basic metrology of the ionizing radiations, the construction of a laboratory and the purchase of equipment at an overall cost not exceeding 1,800,000 gold francs (about £210,000).

(*b*) The raising of the total annual subvention made jointly by the member-States from the present figure of about 400,000 gold francs (£47,000) to 900,000 gold francs (about £105,000).

The intentions of these decisions are that the Bureau International, which has hitherto had to work within limits imposed by a much too narrowly restricted budget, should be better able to fulfil its function as a supra-national standards laboratory and to provide it with reasonable funds for this purpose.

To enable the Bureau International to initiate as early as possible the new section on the ionizing radiations, the Ford Foundation has made a grant of 32,500 dollars to cover the period intervening until the new funds voted by the Conference become payable by the member-States on January 1, 1962. The National Research Council of Canada has temporarily seconded to the Bureau International Dr. C. Garrett, head of the Ionizing-Radiations Section of its Division of Applied Physics, who will take charge of the new section during this same period. His duties will include detailed planning of the ionizing-radiations laboratory and the ordering of its equipment; he will also advise the director of the Bureau International des Poids et Mesures on the selection and appointment of staff for the new section.

Despite its partial revision in 1921, the Convention du Mètre retains many clauses, suited both scientifically and politically to nineteenth-century circumstances, which tend to hinder adaptation of the organization to the rapidly changing and complex needs of scientific metrology and international relationships in the twentieth century. Accordingly, a special commission of the International Committee had been working on the draft of a revised treaty since the last Conference in 1954 and this draft had been sent to Governments for comment some months ago. The scientific clauses were easy to settle, but it was soon realized during the eleventh Conference that there was no hope of securing immediate and unanimous approval of the political and administrative clauses of a modernized treaty by the various delegations, so the matter was left to proceed by negotiation through diplomatic channels.

The fundamental scientific resolution unanimously adopted by the Conference re-defined the metre as equal to 1,650,763·73 vacuum wave-lengths of the orange line (6058 Å., spectroscopic designation $2p_{10}$–$5d_5$) emitted by the krypton atom of mass 86. The source of this radiation, recommended in October 1960 by the International Committee, is an Engelhard type of krypton-86 hot-cathode discharge lamp[1,2] enclosed in a cryostat maintained at the triple-point of nitrogen (about 63° K.). The specification of the conditions of excitation and observation ensures a reproducibility of the new optical metre to about 1 part in 100 millions, and there are good expectations of improving on this very soon. Recent experimental work at the National Research Council (Ottawa) and the National Bureau of Standards (Washington) indicates that continuity of the unit metre distance is maintained to the accuracy with which it was represented by the separation, at 0° C., of two parallel defining lines engraved on the prototype platinum–iridium bar, namely, 1 part in 4 millions. Moreover, according to the new definition, the angstrom unit (Å.) becomes equal to 10^{-10} metre exactly[3].

It is interesting to digress here by noting that the Weights and Measures Bill now before Parliament proposes to define the yard as equal to 0·9144 metre exactly. The same value has already been adopted by the National Physical Laboratory (and five other national laboratories) for non-metric measurements of length in science and technology[4]. If the Bill is enacted, the yard for all purposes in Britain will therefore be effectively based on the new primary wave-length standard through the adopted relationship with the metre.

Among other scientific resolutions adopted by the Conference are the following relating to units and standards of measurement:

(1) Ratification of the improved astronomical second of time[5], with a rider urging standards and other laboratories to proceed as actively as possible with the work to establish a second based on an atomic frequency standard.

(2) Provisional retention of the Potsdam gravimetric system[6] for use in physics until the results of certain absolute determinations of gravity now in progress are made known.

(3) Approval of a detailed list of the international system of units[7] (designation *SI*) founded on six basic units (metre, kilogram, second, ampere, degree Kelvin and candela).

(4) Approval of a new draft of the International Temperature Scale (1948)[8], henceforth to be known as the International Practical Scale of Temperature (1948).

(5) Inviting the International Committee to study, and if possible resolve, the confusing situation due to the difference of 28 parts in a million between the cubic decimetre and the litre[9].

The eleventh General Conference was by far the most interesting and stimulating of the three Conferences held since the end of the Second World War. Besides giving official recognition to a fundamental change in the definition of the metre, some very difficult administrative and financial questions were resolved in such a way as should engender a new look for international scientific metrology during the next decade.

This article is published by permission of the director of the National Physical Laboratory, who led the United Kingdom delegation at the eleventh General Conference.

H. BARRELL

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DOMESTIC FOOD CONSUMPTION AND EXPENDITURE IN BRITAIN

THE recently issued annual report of the National Food Survey Committee for 1958 is the ninth in the series of surveys, begun in 1950, of food consumption and expenditure on food in private households in the United Kingdom[*]. During 1958, 8,611 records were compiled from 827 polling districts in England, Wales and Scotland. The survey methods remained unchanged. Information was collected on all foods purchased or brought into the household during one week, but, as before, soft drinks, sweets, ice-cream and snacks were not included, and full details of meals taken away from home not obtained; an allowance has been made for these when the nutritive value of the diet is assessed. Records are no longer sought of changes of larder stocks, these being largely unnecessary for the low-income groups in which daily shopping is traditional. In the higher-income groups bulk purchasing of some items is more frequent, and a large number of households would have to be surveyed to obtain a statistically correct record.

While the survey is thus incomplete on the food consumption side, it provides much valuable information on the expenditure on food and eating habits of the community, set out in a series of well-presented tables which enable the expenditure on many types of foods by families differing in social class, composition and location to be compared.

For Britain as a whole, weekly expenditure on food (with allowances for free and home-grown food) rose from 29*s.* 1*d.* per head per week in 1957 to 29*s.* 4*d.* in 1958. This small increase is attributed to higher seasonal prices. Since 1954, when rationing ceased, the percentage rise in expenditure on food has increased year by year. The report notes that "the average calorie intake of the population has reached such a level that there is little scope for increases in the quantum of purchases except through improvements in quality, service, processing and packing or through increased wastage".

Little change has occurred since 1954 in expenditure on the broad food groups, but within these groups there has been a significant increase in purchases of processed foods such as cakes and biscuits and certain kinds of processed meat, fish, vegetables and fruit. The consumption of butter continues to rise and that of margarine to fall. The steady decrease in expenditure on bread has halted and shows a slight rise, mainly due to the purchase of dearer types of bread.

On the average there has been little change in the nutritive value of the average British diet since 1954. When compared with the allowances recommended by the British Medical Association, it is found to be satisfactory. Supplies of nearly all the nutrients are, however, greater in the higher- than in the lower-income groups. In the latter, supplies of protein are lower than the British Medical Association's recommended allowance for households, with total incomes of less than 230*s.* a week, and old-age pensioners may be somewhat short of iron. No data are available indicating that these deficiencies affect the health of the groups in question.

A chapter on geographical differences in food purchasing contains some interesting information. For example, in Scotland expenditure on cakes and biscuits was 36 per cent more than the average, and that on flour 40 per cent less than the average. Welsh households spent 30 per cent more than the average on flour, 60 per cent more on butter and 12 per cent less on cakes and biscuits.

Two new special studies were included in the 1958 survey. One deals with the effect on the household diet of the housewife's employment, which causes a change in the whole pattern of eating habits with more meals and snacks taken away from home. The results of this study were inconclusive, because of the difficulty in obtaining information about outside meals, and elaborate statistical treatment was scarcely warranted. The second special study was concerned with the food consumption of households dependent on one woman with no other wage-earner, and here the information collected was disturbing. The diets were in general less than the average in nutritive value, particularly in protein and calcium content, and, in the larger families so dependent, there was also a deficiency in calcium and riboflavin.

While the picture is on the whole satisfactory, the data for larger families check complacency. In households of two adults and two or more children, protein supplies are less than the British Medical Association's recommended allowances, and when there are four or more children the diet falls short of the allowance not only with respect to protein but also in calorie value and calcium and riboflavin content. The fact that these figures are an average for large families of all income groups means that consumption in the lower-income groups in this category is far less than the average for the whole groups of households surveyed.

JOYCE DOUGHTY

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[*] Ministry of Agriculture, Fisheries and Food. Domestic Food Consumption and Expenditure, 1958: Annual Report of the National Food Survey Committee. Pp. vii+185. (London: H.M. Stationery Office, 1960.) 10*s.* net.

IMMUNIZATION IN INDIA

IN a recent issue of the *WHO Chronicle* the possible part that could be played by wide-scale immunization against disease in India, provided outside support is given, is considered (14, No. 11; November 1960).

About 13·5 per cent of India's 408 million inhabitants are infants and children of 4 and under; about 25 per cent are between 5 and 15 years old. Diphtheria, whooping cough and tetanus cause widespread morbidity and mortality in these age groups. Diphtheria—also rife in rural areas—is responsible for between 2,000 and 5,000 admissions every year to hospitals in cities such as Calcutta and Bombay, and no fewer than 84 per cent of the cases are children between 2 and 10 years of age, while 11 per cent are infants. Investigations have shown that tetanus (including that of the new-born) is an important cause of death in rural India. Whooping cough not only causes much suffering but also many deaths from secondary complications.

In 1959, the twelfth session of the World Health Organization Regional Committee for South-East Asia recommended the routine vaccination of children against all three diseases. The major obstacle to this course in India has hitherto been the difficulty of obtaining the necessary vaccines ; local production is inadequate and the cost of importing them prohibitive. It has therefore been decided to expand their local manufacture with help from the World Health Organization and Unicef as a preliminary to a country-wide immunization campaign against these three diseases.

A pilot unit for the production of the vaccines will be set up at the Central Research Institute, Kausali. As soon as practical methods of preparation have been worked out, standardized and tested, other laboratories will be encouraged to adopt the approved methods and start production so that the entire needs of the country can be met step by step. At the same time, epidemiological information on the diseases will be collected from both rural and urban areas and used to plan a practical and economical immunization programme for the whole country. The vaccines will be manufactured at Kausali in accordance with procedures recommended by the World Health Organization, and a consultant from that Organization will be assigned to the project. Equipment, apart from that already available in the country, will be supplied by Unicef.

Two workers from the Kausali Institute are at present in Australia studying vaccine production and immunology, including the production of triple vaccine against diphtheria, whooping cough and tetanus. On their return to India they will work with the staff of the special vaccine production unit. Another worker from the Institute will be trained overseas at a later date. It is expected that the Institute will be able to produce 4,000,000 ml. of vaccine a year to begin with, and within three years will reach the target of 7,000,000 ml. a year.

DISTRIBUTION OF CERTAIN POPPY-FUMARIA ALKALOIDS AND A POSSIBLE LINK WITH THE INCIDENCE OF GLAUCOMA

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GLAUCOMA, suddenly affecting large numbers of people and associated with dropsy ('epidemic dropsy'), has occurred sporadically in India in epidemic form, and these epidemics were eventually traced[1] to the contamination of cooking oils with the seed-oil of the common prickly yellow poppy, *Argemone mexicana* L. Since epidemic glaucoma in India was caused by a plant poison, it was suggested[2] that endemic (primary) glaucoma of unknown ætiology, occurring in other parts of the world, might also result from some toxic cause. Endemic glaucoma, empirically recognized for centuries, is one of the major causes of blindness, and Duke-Elder[3-5] has repeatedly emphasized that, in spite of extensive researches, its basic cause, mechanism, and radical treatment remain unknown. Glaucoma is symptomless during the greater part of its relentless development, and in its early stages merely shows as an increase in the normal range of fluctuation in the fluid tension of the eye. Only later is the tension permanently raised, accompanied by progressive, perhaps independent, degeneration of the drainage mechanism and nervous structure of the eye[3,6,7]. In spite of facilities for early detection and medical and surgical treatment available in advanced countries, such as the United Kingdom, nearly 60 per cent of cases proceed to total blindness[8].

The seed-oil of *Argemone mexicana* contains the *iso*quinoline alkaloids sanguinarine and dihydrosanguinarine[9], and we[10a,b] have described elsewhere an investigation of the biological effects of argemone oil and of sanguinarine. In particular, the production of experimental glaucoma and dropsy was described, and both these cardinal signs of the human epidemic disease were reproduced in animals for the first time. Sanguinarine, a pure alkaloid, was thus the first known naturally occurring substance successfully used as a tool for the production and study of experimental glaucoma. Eye-tension-raising properties were, however, not restricted to sanguinarine and to argemone oil. Tests on a large group of rabbits established that the closely related alkaloid chelerythrine, and oils or watery extracts of seven other species of poppies also raised eye tension[10]. These observations of ours led others[11] to investigate in rabbits the effects on eye tension of a number of *iso*quinoline alkaloids belonging to various types ; besides sanguinarine, the intravenous injection of codeine, papaverine, narcotine, bulbocapnine, corydine, corybulbine, and chelidonine also produced acute rise of eye tension[11]. Our own experiments were continued[12] in India with rhesus monkeys, as their eyes more closely resemble those of man. Repeated injections of sanguinarine produced dropsy and sustained rise in eye tension, while feeding with argemone leaves, or with seed-oil of either argemone or the Indian fumitory (*Fumaria indica* Pugsl.), produced dropsy and a smaller rise in eye tension.

Sanguinarine is a member of the small benzophenanthridine group of *iso*quinoline alkaloids. Although isolated[13] in 1829 from *Sanguinaria canadensis* L., it had, up to the time the present work was begun in September 1956, been detected in only nine species of poppy-fumaria weeds [*Argemone mexicana* L. ; *Bocconia pearcei* Hutchinson ; *Chelidonium majus* L. ; *Dicranostigma franchetianum* Hook. f. et Thoms. ; *Eschscholtzia californica* Cham. ; *Glaucium flavum* Crantz. (=*G. luteum* Scop.) ; *G. fimbrilligerum* Boiss. ; *Macleaya microcarpa* Fedde (=*Bocconia microcarpa* Maxim.) ; *Sanguinaria canadensis* L.], while its occurrence in three other species (*Bocconia cordata* Willd. ; *Dicentra spectabilis* L. ; *Stylophorum diphyllum* Nutt.) was not confirmed[14,15]. While our work was in progress, its presence in *Glaucium corniculatum* Curt. was recorded by Slavík and Slavíková[16], and, since our work was completed in March 1958, the painstaking researches of Slavík and his collaborators have demonstrated its presence in *Dicranostigma franchetianum* Fedde[17], *Dicentra spectabilis* L.[18], *Glaucium flavum* var. *fulvum* Fedde[19], *G. vitellinum* Boiss. et Buhse[20], *G. elegans* Fisch. et Mey.[21], *Argemone alba* Lestib.[22], *Meconopsis aculeata* Royle, *M. rudis* Prain, *M. paniculata* Prain, *M. betonicifolia* Franch., *M. horridula* Hook. f. et Thoms., and *M. latifolia* Prain[23]; a few of these observations confirm, and they are in general complementary to, those recorded in the present article.

The meagre information hitherto available on the distribution of sanguinarine may be attributable to difficulties in adequate extraction and identification, since sanguinarine and some of the other alkaloids with which we are concerned show somewhat unusual partition properties and are not detected by several of the common 'general' reagents for alkaloids. Fortunately, these alkaloids fluoresce brilliantly in distinctive colours in filtered ultra-violet light, and the fluorescences show individual and characteristic changes in colour with change of *p*H. They could be separated either by paper chromatography or by paper electrophoresis and detected even in exceedingly small quantities by their fluorescences, R_F values, and relative speeds of migration. Paper chromatographic runs were made by descending flow on Whatman No. 1 paper using *n*-butanol/glacial acetic acid/water (63 : 10 : 27 or 100 : 10 : 30); second dimensional runs were made with a similar mixture (85 : 1 : 14)[24]. In filtered ultra-violet light, sanguinarine spots fluoresced with a bright orange colour, chelerythrine and coptisine spots with a golden yellow colour, and berberine spots with a brilliant yellow-green colour; touching the spots on the paper with a glass rod previously dipped into *N* sodium hydroxide produced characteristic reversible changes in fluorescence. Paper electrophoresis runs were made on Whatman No. 54 strips, 12·5 cm. wide, moistened with 10 per cent formic acid[25], with a potential difference of 1,000 V. and a potential gradient of 20 V./cm., using sanguinarine, or berberine, as marker.

Herbs of the families Papaveraceae and Fumariaceae were gathered from the Royal Botanic Gardens, Kew, the University Physic Garden, Oxford, and from India. Different parts of each plant were ground in small volumes of glacial acetic acid, which was found to be the best extraction medium. The alkaloids in such extracts were easily and clearly separated by paper chromatography and paper electrophoresis[10c]. From a few drops of plant extract, ten or more fluorescent spots were frequently obtained by either method. Chromatographically separated fluorescent spots were eluted and re-spotted for electrophoresis for confirmation of their basic nature and identity. With these very sensitive methods, numerous fluorescent alkaloids were readily separated from even a few milligrams of plant material in suitable cases, and the results are recorded in Table 1 for the distribution of four of the principal alkaloids (berberine, coptisine, chelerythrine and sanguinarine) between the roots, stems, leaves, flowers, capsules, and seeds—or such of these parts as were available to us—of the species examined. Separation of alkaloids present in gram quantities of plant material could also be effected on small alumina columns developed with benzene, acetic acid and ethanol[24].

Sanguinarine was found in 49 out of the 53 available species of poppy-fumaria weeds and usually in several different morphological parts of the plant (Table 1). Far from being restricted to only a few genera of poppy-fumaria plants as previously believed[16], it was perhaps the commonest fluorescent alkaloid detected among these weeds. It was almost invariably found in the leaves and stem. When the same species was collected from different localities, or at different stages of its growth, small qualitative and quantitative variations were noted in the alkaloidal content. Chelerythrine was found less frequently than sanguinarine; it was common in certain genera and rare in others. Berberine, though of fairly frequent occurrence in poppy-fumaria plants, was restricted to certain genera with rare exceptions. Sanguinarine and chelerythrine were not detected in a few specimens from the phylogenetically related family Berberidaceae, in which berberine was abundant in the stems yet absent from the leaves. Canadine was rarely detected. On the other hand, the closely related protoberberine alkaloid coptisine was very often seen. The more widespread occurrences of sanguinarine and coptisine that have emerged from the present work are of great interest, but perhaps not altogether surprising, as protopine is said to be without doubt the most widespread of all alkaloids and to have been found in all papaveraceous plants which have been exhaustively examined[15,27], and sanguinarine, coptisine, and protopine probably share, according to widely accepted views on alkaloid biosynthesis[28,29], a common protoberberine precursor.

We have found sanguinarine in the root, stem and leaves of the 'opium' poppy (*Papaver somniferum* L.) but not in the capsule or in the seeds. It is presumably not present in opium as it has not been noticed in spite of intensive studies on the alkaloids of opium[27] during the past hundred years. In Persia young opium plants pulled up to prevent excessive cropping are used as pot herbs[30] and are fed to cattle. Although we failed to detect sanguinarine in the specimen of *P. somniferum* seeds examined, the culinary use of poppy seeds (maw seed, *P. somniferum* L. var. *nigrum* DC.) should be viewed with suspicion, as we examined a poppy-seed cake purchased in a London supermarket and found it to contain several fluorescent bases and a small but quite unmistakable proportion of sanguinarine. Poppy seeds and their oil are extensively used in bread and confectionery in France, south-east Europe, and western Asia, especially by Jews, who, as a people, have a notably high incidence of glaucoma[31,32]. Insidious sanguinarine poisoning could occur from various species of poppy seeds, especially those of *P. rhoeas* L., which have a tendency to adhere to cereals to the extent of up to 10 per cent. These seeds are also fed to poultry as 'tail-corn', and probably transmit their alkaloids to eggs. Through the courtesy of Dr. M. E. Langham and Dr. J. G. Dobbie of the Medical Research Council Ophthalmological Research Unit, Institute of Ophthalmology, we have been able to examine the eggs from hens forcibly fed with sanguinarine, using the hydrolytic-extractive procedure described in the following article; we found sanguinarine in the whites and traces in the yolks along with other fluorescent bases, none of which was found in control eggs.

Sanguinarine was found in nearly all the 53 species of poppy-fumaria weeds which we examined. These species are distributed among 14 genera, and this suggests that sanguinarine is probably present in many more of the 675 species[33] of this vast alkaloid-rich family with its world-wide distribution. In view

Table 1. MORPHOLOGICAL DISTRIBUTION OF FOUR *iso*QUINOLINE ALKALOIDS IN 53 SPECIES OF POPPY-FUMARIA WEEDS

Plant	Berberine						Coptisine						Chelerythrine						Sanguinarine					
	r	*s*	*l*	*f*	*c*	*sd*	*r*	*s*	*l*	*f*	*c*	*sd*	*r*	*s*	*l*	*f*	*c*	*sd*	*r*	*s*	*l*	*f*	*c*	*sd*
Papaveraceae																								
Argemone alba Lestib.		+	+		+	+		−	−		−	−		−	−		−	−		?	+		+	+
A. grandiflora Sweet	?	−	−	−	+		+	+	+	+	+		+	−	−	−	−		+	?	?	+	+	
A. mexicana L. (London)		+	+		?	+		+	+		+	+		−	−		−	−		+	?		+	+
A. mexicana L. (Bombay)	+	+	+	?	+	+	−	−	−	−	−	−	+	−	−	−	−	−	+	+	+	?	+	+
A. mexicana L. (Bombay seedlings)	+	+	+				−	−	−				?	?	−				+	+	?			
A. mexicana var. *ochroleuca* (Sweet) Lindl.		+	+		+	+		+	+		+	+		−	−		−	−		+	+		+	+
A. platyceras Link et Otto		−	−		+	−		+	−		+	+		?	−		−	+		+	−		+	+
A. platyceras var. *rosea* Coult.		+	+		+	+		+	+		+	+		−	−		−	−		+	?		+	+
Chelidonium majus L.	+	+	?		+	+	+	+	+		+	+	+	?	+		−	−	+	+	?		+	?
Dicranostigma franchetianum Fedde	−	−	−		−		+	+	+		+		+	?	+		+		+	+	+		+	
D. lactucoides Hook. f. et Thoms.			−		−	−			+		+	−			+		+	−			+		+	+
Eschscholtzia caespitosa Benth.		−	−			−		+	+			+		+	−			+		+	?			+
E. californica Cham. (London)	−	−	−			−	+	+	+			?	+	−	+			+	+	−	+			+
E. californica Cham. (N. India)	−	−	−				−	−	?				+	−	+				+	+	+			
E. californica var. *alba* Fedde		−	−		−	−		−	−		+	?		−	−		+	+		+	?		+	?
E. lobbii Greene	−	?	−		−	−	+	−	+		−	+	+	−	?		−	+	+	?	+		+	+
Glaucium corniculatum var. *rubrum* S.M.		−	−		−	−		+	+		+	+		+	+		+	−		+	+		+	?
G. flavum Crantz		−	−			−		−	−			−		−	−			−		?	+			+
Hypecoum grandiflorum Benth.		−	−		−	−		−	−		−	−		−	−		−	−		−	+		+	+
Macleaya cordata R.Br.	−	−	−	−		−	+	+	−	−		−	+	+	+	+		+	+	+^{e}	+^{ed}	+		+
M. microcarpa Fedde		−	−					+	+					+	+					+	+			
Meconopsis betonicifolia Franch.			−						−						−						+^{e}			
M. cambrica Vig.		−	−					−	−					−	−					?	−			
M. cambrica var. *amintica*		−	−		−	−		−	+		−	−		−	−		+	−		+	+^{e}		−	−
M. dhwojii G. Taylor		−	−					−	−					−	−					+^{e}	+^{e}			
Papaver aculeatum Thunb.	−		−		−	−	−		?		−	−	−		−		−	−	+^{e}		−		−	−
P. atlanticum Ball	−		−		−	−	−		+		?	−	−		−		−	−	?		+^{e}		−	−
P. commutatum Fisch. et Mey.		−	?	−	−	−		−	−	−	?	?		−	−	−	?	−		−	+	−	−	−
P. dubium L.	+	+	+		+	−	+	+	+		−	−	−	−	−		−	−	?	−	+^{e}		−	−
P. glaucum Boiss. et Hausskn.					−	−					+	+					−	−					+^{e}	−
P. heldreichii Boiss.					−	−					?	−					?	−					+	−
P. hybridum L.	+	−	+	+			+	+	+	+			−	−	−	−			+	?	?	?		
P. monanthum Trautv.			−		−	−			−		+	+			−		?	?			?		+	?
P. nudicaule L.	−	−	−	−	−	−	+	−	+	+	?	−	−	−	−	−	?	−	+^{e}	?	+	−	+	−
P. orientale L. (Oxford)	−	−	−		−	−	−	−	−		−	−	−	−	−		−	−	+	−	−		+	−
P. orientale L. (London)		−	−		−	−		+	−		+	+		−	−		−	−		−	−		−	−
P. pavoninum Mey.		−			−	−		+			+	−		−			?	−		−			−	−
P. pilosum Sibth. et Sm.	−	−	−		−	−	+	+	+		+	−	−	−	−		?	−	+^{e}	+	+^{e}		?	−
P. rhoeas L. (N. England)		−	−	−	−	−		?	?	−	?	−		?	?	−	?	−		+	+	−	+	−
P. rhoeas L. (Oxford)	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	+^{ed}	−	?	?	−	+
P. rhoeas L. (London)		−	−	−	−	−		+	+	−	+	+		−	−	−	−	−		?	?	−	?	?
P. setigerum DC.					−	−					+	?					−	−					?	−
P. somniferum L. var. purple (Oxford)	−	−	−				+	+	+				−	?	−				+	+^{ed}	+			
P. somniferum L. var. white (Oxford)	−	−	−				−	+	−				?	?	?				+^{e}	+^{e}	+^{e}			
P. somniferum L. var. red (N. India)	−	−	−	−			−	−	+	+			−	−	−	−			+	+	−	+		
P. somniferum L. var. red (Anatolia)		−			−	−		−			−	+		−			−	−		−			−	−
P. triniifolium Boiss.	−	−	−		−		+	+	+		+		−	−	?		−		+^{e}	+	?		−	
Roemeria rhoeadiflora Boiss.	−	−	−		−		+	+	+		+		−	−	−		−		−	−	−		−	
Stylophorum diphyllum Nutt.		−	−					+	+					+	−					+	+^{ed}			
Fumariaceae																								
Corydalis chaerophylla DC.		?	+					+	+					−	+					+^{e}	+^{e}			
C. cheilanthifolia Hemsl.		+	+		+	?		+	+		+	+		−	?		−	−		+	+		+	?
C. glauca Pursh		−	+					+	+					−	?					?	+^{e}			
C. glauca var. *alba*		−	−		−	−		+	+		?	?		−	−		−	−		+	+		+	+
C. govaniana Wall.		+	−					+	+					−	−					?	?			
C. lutea DC.	+	+	+		+	−	+	+	+		+	−	−	−	−		−	−	?	?	?		−	−
C. meifolia Wall.		?	+					+	+					−	−					−	−			
C. thyrsiflora Prain		−	−					?	?					−	−					−	−			
Dicentra formosa Walp.		−	−					−	−					−	−					+	+^{ed}			
Fumaria indica Pugsl. (N. India)	−	−	−		−	−	+	+	+		+	+	−	−	−		−	−	+	+	−		+	+
F. indica Pugsl. (bazaar seeds)						?						+						?						+^{ed}
F. micrantha Lag.		−	−			−		+	+			+		−	−			−		+	+			+^{e}
F. officinalis L.	−	−	−			−	+	+	+			+	−	−	−			−	+	+	+			+^{d}
F. vaillantii Loisel		−	−					+	+					−	−					+	+			
Berberidaceae																								
Berberis chitria D. Don		+	−					−	−					−	−					−	−			
B. verruculosa Hemsl. et E. H. Wils.		+	−					−	−					−	−					−	−			
B. vulgaris L.		+	−					−	−					−	−					−	−			

r, roots; *s*, stem; *l*, leaves; *f*, flower; *c*, capsule; *sd*, seeds; +, alkaloid present; −, alkaloid absent; ?, presence of alkaloid not definitely confirmed; blank space denotes part not available for investigation; +^{d}, presence of alkaloid confirmed by two-dimensional paper chromatography; +^{e}, presence of alkaloid confirmed by paper electrophoresis; +^{ed}, presence of alkaloid confirmed both by paper electrophoresis and by two-dimensional paper chromatography. Approximate R_F values on Whatman No. 1 paper in butanol/glacial acetic acid/water (100 : 10 : 30): berberine, 0·59; coptisine, 0·44; chelerythrine, 0·54; sanguinarine, 0·48.

of the much wider distribution of this toxic alkaloid than has hitherto been suspected, the attendant risks of contamination of milk and other foodstuffs become more obvious. Many of the traditional uses of poppy-fumaria products in various parts of the world, particularly their inclusion in medicaments, are dangerous and should be carefully reviewed. It is obvious that, while sanguinarine was the first pure substance observed to produce glaucoma experimentally[10], many other substances can probably do so in varying degrees[10,11], and the possibility that endemic primary glaucoma results from chronic poisoning with some substance or another should be borne in mind. Such chronic poisoning could arise by the innocent ingestion of the responsible agent or could be the unrecognized side-effect of drugs used for legitimate therapeutic purposes. The present communication reviews the distribution of sanguinarine and related alkaloids in Nature, and points to the likelihood of their transmission to man in food and drugs, while milk as a possible vehicle is considered in the following article.

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EXPERIMENTAL TRANSMISSION OF SANGUINARINE IN MILK: DETECTION OF A METABOLIC PRODUCT

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DIETARY factors are known to affect the quality of milk, and taints and off-flavours are often caused by the ingestion by cows of a variety of weeds, perhaps the most familiar being the taint acquired by cream and butter made from the milk of cows that have eaten cruciferous plants. Milk, however, probably always contains, in trace amounts derived from the pasture or other feeding-stuffs, undesirable substances that do not declare their presence as frankly as those that produce taints. Our present concern is with the possibility that toxic poppy-fumaria alkaloids, and sanguinarine (I) in particular, may be transmitted to man in milk from cattle grazing on pastures containing poppy-fumaria weeds, as we have previously suggested[1], and we wish to report some model experiments in that connexion. A striking example of the transmission of toxic alkaloids in milk has recently been given by Schoental[8], who found that the administration of certain *Senecio* alkaloids to the lactating rat produced fatal liver lesions in the young without having any apparent effects on the mother or on her lactation, and she further made the suggestion that various liver disorders in childhood may be the result of poisoning by milk.

Evidence supporting the possibility of transmission of argemone alkaloids in milk was first obtained in India when argemone leaves were added to a goat's diet. Some toxic substance was obviously absorbed and transmitted, for feeding the goat's milk to four rhesus monkeys produced a rise in their eye-tension. A further experiment was made to determine if still higher levels of eye tension could be reached and maintained by reducing the cysteine content of the poisoned goat's milk. This was based on the human epidemiological records which showed that argemone poisoning was more severe in poorly fed communities and on our earlier animal experiments in which cysteine had diminished or prevented the toxic effects of sanguinarine[1a]. Four monkeys were fed on a synthetic diet low in cysteine, prepared with the casein-fat portion of the toxic goat milk; in two animals the basic tension rose to much higher levels and decreased only slightly after about eight weeks. The results obtained with these monkeys confirmed the theoretical possibility that milk can transmit poisons that raise eye tension[1b]. Argemone leaves contain traces of sanguinarine together with other *iso*quinoline alkaloids (ref. 3 and preceding article), and it is possible that other alkaloids, also secreted in the goat's milk, contributed to produce the rise in eye tension observed in the above experiments. The goats, in spite of producing milk toxic to monkeys, showed only a slightly raised eye tension, and, as a species, goats are known to be resistant to certain other poisons[4].

In the experiments now to be described milk was examined by the following methods: (1) *Dialysis*. The milk was diluted, brought to the mean isoelectric point of its proteins, and dialysed through 'Cellophane'. The dialysate was concentrated under reduced pressure, acidified with acetic acid, and continuously extracted with chloroform for several hours. The aqueous phase was then made alkaline with sodium hydroxide solution, re-extracted continuously with chloroform, and finally with ether. The extracts were evaporated, and the combined residues were spotted on paper for chromatography or electrophoresis in the manner described in the preceding communication. (2) *Acid hydrolysis*. The milk was heated with an equal volume of 3 *N* hydrochloric acid at 100° C. for 4 hr., and the cooled mixture was then extracted continuously with organic solvents at both acid and alkaline *p*H as described in the preceding method. When sanguinarine was artificially added to control milk, the quantities afterwards recovered by dialysis were small, but acid hydrolysis led to greater recovery.

Lactating rabbits (2·5–3·0 kgm.) were separated from their young and injected subcutaneously in the flank with 4–8 mgm. of sanguinarine chloride in aqueous solution. At varying intervals during the next 4 hr., 5–10 units of oxytocin were injected, and the rabbits were milked by intermittent suction and massage. The milk was then processed as described above, and the extracts were examined by paper chromatography and paper electrophoresis. In four out of ten samples of rabbit milk obtained after injections of sanguinarine the alkaloid and a green-fluorescing metabolic product were detected by both methods. Several other weaker fluorescent spots, presumably representing other metabolic products, were also seen. All these were absent from control experiments with normal rabbit milk. The properties of the green-fluorescing metabolic product are discussed here.

With the assistance of Dr. D. R. Bangham, we succeeded in separating and detecting sanguinarine in as little as 1–2 ml. of the milk of rabbits injected with this alkaloid. The milk was electrophoretically differentiated on a column of 'ethanolysed' cellulose[5] in phosphate buffer, and collected serially in 50 fractions. Sanguinarine could only be detected after hydrolysis of the samples, and only in the albumin fractions. Many alkaloids are known to be strongly adsorbed on to serum albumin, and this was found to be the case for sanguinarine. After injecting sanguinarine into rats, cats and monkeys, 1 ml. samples of their plasma were collected and separated electrophoretically. As with the milk, sanguinarine was only found after acid hydrolysis and only in those fractions which contained serum albumin. Although present in most of their viscera, the maximum recovery of sanguinarine was from the liver.

In collaboration with Dr. A. H. Gordon we found that sanguinarine was converted in the liver into the green-fluorescing metabolic product. Sanguinarine chloride (1·8 mgm. in 5 ml. normal saline) was added to blood (50 ml.) which was mechanically circulated for 4 hr. through the isolated liver of a rat[6]. Blood samples were taken after 10, 30 and 60 min. and at the end of the experiment. All samples were hydrolysed with hydrochloric acid in the manner described above, and the hydrolysis mixtures were made alkaline with sodium hydroxide and extracted with chloroform. The residues from the chloroform extracts were taken up in about 0·5 ml. of chloroform-glacial acetic acid and spotted for electrophoresis on paper in 10 per cent formic acid. No sanguinarine was found at all, but the green-fluorescing substance was present in the 4-hr. specimen and there may have been a trace in the 60-min. specimen.

Properties of the Green-fluorescing Metabolic Product

This substance was best obtained from the urine of rabbits injected with sanguinarine chloride (I). Two rabbits were kept in metabolism cages and received injections of sanguinarine chloride (8 mgm.) daily for 5 days a week for 6 weeks and the urine was collected. The urine (2·5 l.) was acidified with concentrated hydrochloric acid (200 ml.) and heated on the water-bath for 2 hr. with the addition of an anti-foam. The cooled mixture was basified with sodium hydroxide solution and extracted with chloroform (about 1·5 l.). The emulsified organic phase was shaken with water until neutral and then stirred with anhydrous sodium sulphate until a clear chloroform solution was obtained. This process of extracting the hydrolysed urine was repeated four times. The residue obtained on evaporation of the combined chloroform extracts was applied to a column of alumina (activity I/II) (100 gm.), and eluted with benzene–acetic acid. The first fraction was shown by paper electrophoresis to contain the green-fluorescing material and several other fluorescent substances; none of these was observed on similar treatment of the urine from control rabbits receiving the same diet. The fraction containing the green-fluorescing substance was further purified by solution in benzene and extraction with 20 per cent hydrochloric acid. The acid solution was basified and extracted with chloroform. The chloroform solution was shown by paper electrophoresis to contain the green-fluorescing substance, and the material was chromatographed in dry benzene on alumina (10 gm.) as before. The first fraction, coming off the column as a blue-violet fluorescing solution, deposited a few minute colourless needles on evaporation and contained the substance sought as the sole fluorescent material (green on paper electrophoresis in acid solution); it was also present in the second fraction together with a substance showing a blue fluorescence on the acid paper electrophoresis strip, but none was present in subsequent fractions, which contained still more fluorescing substances (violet, blue) as shown by paper electrophoresis. The green fluorescing sub-

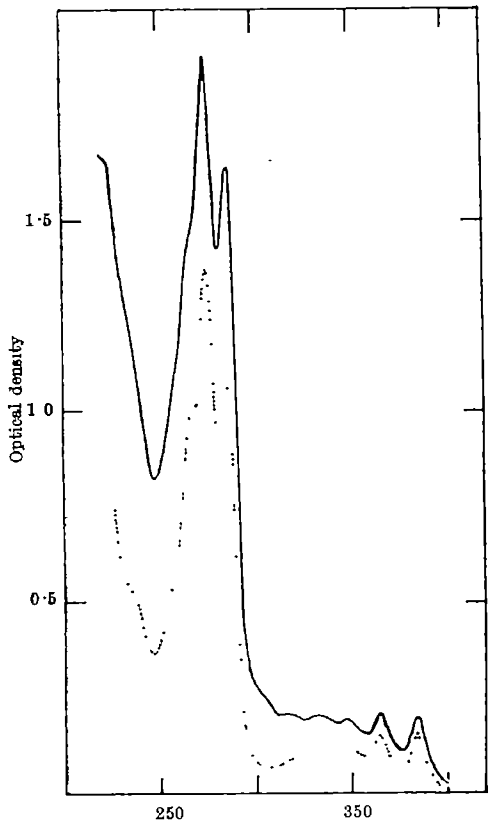
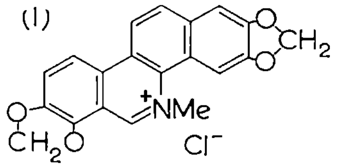


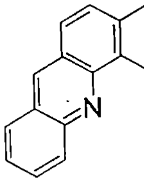
Fig. 1. Ultra-violet absorption spectra in spirit. —— green-fluorescing metabolic product, extracted from paper electrophoresis strip (concentration unknown); . .... 3,4-benzacridine (0 42 mgm./100 ml.)

stance possessed numerous properties identifying it with a high degree of certainty with 3,4-benzacridine (II). A reference specimen of the latter substance was available *via* a hydrogenated derivative formed as a by-product in the preparation[7] of 3,4-benzophenanthridine, and it was later obtained by two unambiguous syntheses[8,9]. The ultra-violet absorption spectrum of the solution obtained by eluting the green-fluorescing metabolic product from the paper electrophoresis strip with ethanol showed a remarkable degree of correspondence with that of 3,4-benzacridine [(II) (Fig. 1); and our colleague Dr G. H. Beaven, who kindly examined the spectra of the two substances for fine structure by the logarithmic cam method[10] in ethanol both before and after the addition of 0·01 *N* hydrochloric acid, reported that the fine structure bands were identical in the spectra of the two substances. The metabolic product and 3,4-benzacridine showed identical behaviour on paper chromatograms and on paper electrophoresis strips, and the fluorescences of the two substances on paper were indistinguishable under acid (green) and alkaline (blue) conditions. Through the courtesy of Prof. R. T. Williams, St. Mary's Hospital Medical School, we were able to compare the fluorescence excitation and emission characteristics of the metabolic product eluted from paper electrophoresis strips with those of 3,4-benzacridine similarly treated using the Aminco-Bowman spectrophotofluorimeter. In 0·1 *N* hydrochloric acid both substances showed activation spectra with maxima at 305 and 390 mμ, exciting fluorescence with a single maximum at 495 mμ; when the solutions were made alkaline with sodium hydroxide a maximum in the activation spectra of both substances appeared at 300 mμ, exciting fluorescence with a maximum at 430 mμ. With a stronger (alkaline) solution of 3,4-benzacridine it was possible to observe another maximum in the activation spectrum at 390 mμ, but light-scattering prevented its observation with the weaker solutions obtained by eluting paper electrophoresis strips.

With so many criteria of identity satisfied, there can thus be little doubt that the metabolic product was indeed 3,4-benzacridine (II), and, if it arose from sanguinarine (and that conclusion seems inevitable), it must have resulted from a profound metabolic alteration for which we know of no close analogy. We have no precise measure of the extent of the conversion of one into the other, but it must be quite small. N-Demethylation calls for no comment, but, in addition, the oxygen functional groups have disappeared and a rearrangement of the carbon skeleton has taken place. Enzymic fission of alkyl aryl ethers[11,12] and metabolic dehydroxylation[11,13] are both well established metabolic reactions, so that the loss of the oxygen functional groups in the present instance may be regarded as extensions of these processes. The rearrangement of the carbon skeleton in passing from sanguinarine chloride (I) to 3,4-benzacridine (II) is possibly also not as bizarre as it would appear at first sight, as there are analogies *in vitro* in the dienone-phenol rearrangement[14], in the anthrasteroid rearrangement[15,16] and in the recently announced conversion of picene into 1,2:5,6-dibenzanthracene[17]. In each of these conversions one part of a condensed ring system exchanges its position of fusion with respect to the rest of the system for an adjacent position, and although only the last of these occurs in a fully aromatic system they presumably all proceed by way of transient spirocyclic intermediates and successions of 1,2-shifts[14,18,19]. A mechanism involving ring-chain tautomerism of an intermediate reduced derivative is, however, not excluded for the metabolic conversion of sanguinarine chloride.

(I)





(II)

It is obvious from these experiments that sanguinarine, together with some of its metabolic products, can be secreted in traces in rabbit milk, and application of the sensitive methods of detection developed in this and in the preceding communication to the milk of goats that have been fed on *Argemone* leaves has now shown the presence of small amounts of sanguinarine in such milk also. Considering the cosmopolitan distribution of poppy-fumaria weeds and the regularity with which sanguinarine occurs in these plants, the likelihood of traces of sanguinarine and of other toxic alkaloids finding their way into milk supplies intended for human consumption must be quite considerable. Under field conditions and in good pastures the unpleasant odour and taste of various *Papaver* spp. render these plants objectionable to animals[19], which frequently show considerable discrimination in their eating habits, but it is otherwise with hay and silage. On the other hand, the common fumitory (*Fumaria officinalis* L.), which is widely distributed and fairly common, is readily eaten by cows though not by goats[20]. Under adverse conditions and in poor neglected pastures cattle are obliged to feed more indiscriminately and the likelihood of alkaloidal contamination of milk in such circumstances must be considerably greater. Whether or not these observations have a bearing on the incidence of endemic primary glaucoma cannot at present be assessed, and they are merely offered as circumstantial evidence to show that regular unintentional absorption of minute amounts of sanguinarine and of other papaveraceous alkaloids by man is feasible.

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MAMMARY CANCER INDUCED BY A SINGLE FEEDING OF POLYNUCLEAR HYDROCARBONS, AND ITS SUPPRESSION

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ONE of the most remarkable experiments in cancer research is the induction of cancer which occurs preferentially in the breast of rats following the feeding of carcinogenic hydrocarbons. The first compound found to incite mammary cancer by this method was 2-acetylaminofluorene (2-AAF) which was incorporated in a diet that was fed to rats for many months[1]. The repeated daily administration of 3-methylcholanthrene (3-MC) to young adult female rats of a special strain induced mammary cancer invariably and rapidly[2,3]; the earliest cancer of the breast was detected by palpation 20 days after beginning the feeding, and within two months every animal had developed multiple tumours of this sort. A newly recognized property[2] of the mammary cancers induced by this method was the dependence of many of the tumours on the presence of hormones, since regression takes place after ovariectomy or certain other endocrine procedures. This is a characteristic that is commonly observed in cancer of the breast in human beings, but which is unique among mammary cancers available in the laboratory.

It will now be shown that a single feeding of polynuclear hydrocarbons to rats caused selective injuries in the recipient rather than total body damage. These lesions include the induction of leukopenia and of cancers of two types, carcinoma of the breast and of the external acoustic duct. Further, established mammary cancers were suppressed in many rats by treatment with certain hormones or by pregnancy.

The induction of mammary cancer following the application of aromatic carcinogens remotely from the breast was first observed by Maisin and Coolen[4] who painted the skin of mice with 3-MC and observed a high incidence of cancer of the breast in addition to cancer of the skin. A single intramuscular injection of carcinogenic hydrocarbons is a classical method of evoking sarcoma in susceptible rodents[5]. An injection of 3-MC into the breast of mice gave rise to sarcoma and adeno- and squamous carcinoma of the mammary glands[6]. A single intravenous dose of urethane gave rise to benign pulmonary tumours in mice[7].

Induction of Mammary Cancer

Female rats of the Sprague–Dawley strain were used in all the experiments, with 10 rats in each experimental group; unless stated otherwise, the animals were age 50 days and weighed 140–150 gm. when the hydrocarbons were fed. They remained free from respiratory diseases.

With the aid of gentle heating, 2 gm. of 7,12-dimethylbenz(a)anthracene (DMBA), or 1·5 gm. of 3-MC was dissolved in 100 ml. of sesame oil. One gram of 2-AAF was dissolved in 10 ml. of ethanol and the solution was diluted to 100 ml. with sesame oil. The solutions were instilled in the stomach through a soft rubber catheter, and only a single dose was administered. The rats were examined frequently for palpable tumours for 150 days and cancers weighing 10 mgm. or more were readily detected. The occurrence of the cancers was dated from the day of feeding the carcinogen. The tumours were confirmed at necropsy when the total number of visible tumours ('active centres') was counted.

In our rat colony two 'spontaneous' mammary cancers have been observed among about 20,000 untreated female rats less than eight months of age. But benign mammary tumours occur very frequently in this strain in senescence[8].

3-*Methylcholanthrene*. A single feeding of 3-MC (100 mgm.) induced cancer in 100 female rats, aged 50–65 days, in 10 consecutive series; no tumours were observed in 20 males treated similarly. The rats suffered a decline in body-weight (total loss 10–15 gm.) for 48 hr. after the feeding, but a gain in weight was observed on day 3. In these series of females, mammary cancer was observed in every rat and squamous carcinoma of sebaceous glands adjacent to the external auditory canal in two of them; no other tumours were found. The earliest mammary cancer was detected by palpation on day 31, and the last was observed on day 130. But in serial histological sections of the inguinal mammary glands of 10 rats, 14 days after a single feeding of 3-MC, carcinomata were found in two of the glands on microscopic examination. The squamous carcinomata of sebaceous glands appeared, respectively, on days 109 and 121.

The influences of age at the time of a solitary feeding, and of the presence of the ovaries, were investigated by feeding a single dose of 3-MC (66·6 mgm./100 gm.) to rats of various ages; half the animals were intact, and the ovaries of the others had been removed at the time of weaning. In the intact rats mammary cancer was induced by feeding the carcinogen at age 23 days and the incidence of tumours rose progressively with age until all rats fed 3-MC at age 50 days developed cancer of the breast (Table 1). There was a decline in the incidence of mammary cancer in rats fed 3-MC at age 75 days, and cancer of the breast developed in only a small number of animals fed 3-MC at age 100–365 days (Fig. 1).

No mammary cancer was observed in 100 rats that had been ovariectomized at age 22 days and fed a single dose of 3-MC thereafter at various ages between 23 and 100 days.

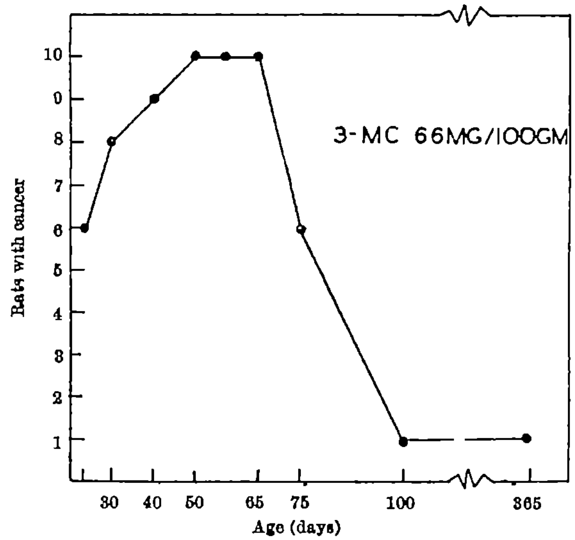

Fig. 1. Incidence of mammary cancer in groups of ten rats fed 3-MC once only. The abscissa indicates the age when the compound was fed

Table 1. INDUCTION OF MAMMARY CANCER BY INTRAGASTRIC INSTILLATION OF POLYNUCLEAR HYDROCARBONS AT 50 DAYS OF AGE

Compound	Dose (mgm.)	No. with cancer*	Appearance of palpable tumours (days)			Active centres
			range	median	mean	
DMBA	20	10	28–59	41	42·8±10†	6·8
DMBA	15	10	34–70	49	48·5±11	3·0
3-MC	100	10	31–86	50	51·3±16	2·8
3-MC	50	8	35–121	56	61·4±30	1·1
3-MC	25	7	34–54	49	47·5±9	2·4
3-MC	10	3	45–129	52	75	1·3
3-MC	5	2	50 and 133	—	—	1·0
3-MC	2	1	48	—	—	1·0
2-AAF	100	3	43–73	53	56·3	1·0
2-AAF	50	8	51–79	59	63	1·0

* There were ten rats in each group. The animals were observed for 150 days.
† Standard deviation.

The optimal dose of 3-MC for the induction of mammary cancer after a single feeding was 100 mgm.; this dose induced 1–4 active centres in each rat. A single dose of 3-MC (2 mgm.) induced mammary cancer in one rat and there was a progressive increase in the rate of tumour incidence and in the number of active centres with augmentation of the dose until the optimal amount was reached (Table 1).

A large portion of 3-MC which is fed passes through the alimentary tract. After a large dose the fæces were highly fluorescent. Our colleagues, Dr. Flesher and Dr. Sydnor, fed a single dose of tritium-labelled 3-MC (10 mgm.) to rats and recovered about 90 per cent of the administered radioactivity in the fæces, where significant quantities were detected for 7–11 days following the single feeding[9].

Trace amounts of 3-MC are sufficient to induce mammary cancer. Compressed, cylindrical pellets of known weight were implanted in the spleen of 18 rats; neoplasms did not arise in the spleen but three of the animals developed mammary cancer at 105, 148 and 199 days respectively. At necropsy, the pellets were removed from the spleen, blotted, heated to 100° for 24 hr. and re-weighed. No decrease in weight of the 3-MC pellet occurred during the long sojourn in the spleen. In one of the experiments wherein a rat developed mammary cancer at 148 days after implantation of 3-MC in the spleen, both the original and final weight of the pellet was 26·8 mgm. An amount of 3-MC too small to be detected on our balance had induced mammary cancer. Shear[5] observed formation of sarcoma in muscle after implanting therein a pellet of cholesterol containing dibenz(a,h)anthracene (0·4 μgm.).

7,12-*Dimethylbenz*(*a*)*anthracene*. A single feeding of DMBA was highly effective in the induction of mammary cancer in female rats. DMBA (1 mgm.) induced mammary cancer, and there was a progressive rise in the incidence of cancers with increased dosage until the optimal dose was reached (Fig. 2). The optimal dose of DMBA (dissolved in sesame oil, 1 ml.) for induction of mammary cancer by a single feeding was 20 mgm. For: (1) every rat survived; (2) mammary cancer developed in 40 consecutive rats without exception; (3) the first cancer was detected by external examination at 28 days and all the rats had visible tumours of the breast before 60 days; (4) the number of active centres in individual rats varied from 3 to 21, the average being 6·8. DMBA (15 mgm.) induced mammary cancer in every rat after a single feeding, but there were fewer active centres than in rats which had received 20 mgm. (Table 1). All 10 rats which were fed DMBA (50 mgm.) died 2–28 days afterwards.

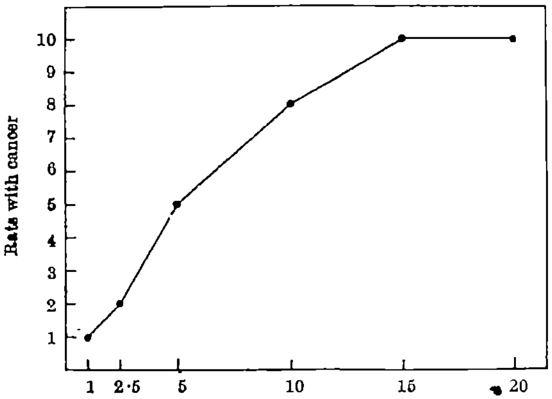

Fig. 2. Incidence of mammary cancer in groups of ten rats fed DMBA once only

It is known that the application of large amounts of 3-MC to the skin of mice causes leukopenia[10]. The leukocytes of the blood of rats were counted in quadruplicate before and at frequent intervals following a single feeding of DMBA (20 mgm.) to rats 50 days old. There was no change in their number for 24 hr., but leukopenia developed shortly thereafter and it was long-lasting (Fig. 3). The steady increase in body-weight of the young rats was interrupted for two days but growth recommenced thereafter (Fig. 3). But the sexual rhythm, demonstrated in the regular recurrence of œstrus cycles, was not delayed by feeding the carcinogen; œstrus appeared every 4·15 days both in DMBA-fed and in untreated control rats. It is clear that DMBA induced cancers and leukopenia and also caused a temporary cessation of body-growth, but brought about no significant decrease in the production of gonadotrophin by the hypophysis or in function of the ovary.

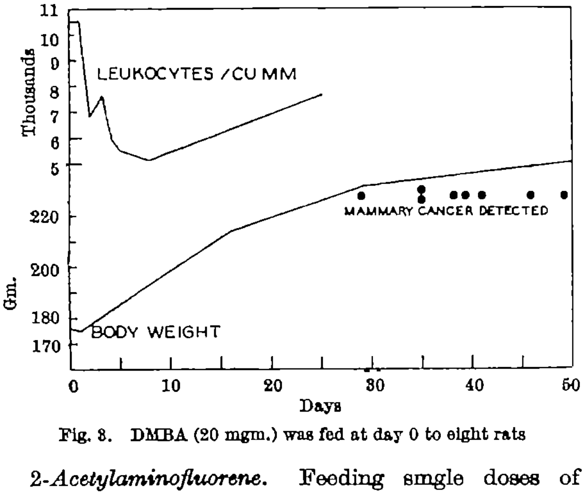

Fig. 3. DMBA (20 mgm.) was fed at day 0 to eight rats

2-*Acetylaminofluorene*. Feeding single doses of 2-AAF was a relatively inefficient method of inducing mammary cancer (Table 1). Twenty rats (50 days old) were fed an alcohol-containing solution of 2-AAF (50 or 100 mgm.) and six of them developed cancer of the breast within five months; no other tumours were observed. The chief interest of this compound was in its anæsthetic effects which were apparent after doses of 75–100 mgm. Due to its limited solubility in oil, 2-AAF was usually given in two divided doses separated by a 4-hr. interval. The rats became drowsy within 1 hr. after the first dose and were asleep within 2 hr. after the second feeding. They remained unconscious and did not move their position in the cage for 24 hr., but most of them woke within a few hours thereafter. Control rats fed sesame oil containing 10 per cent ethanol were not anæsthetized. Moreover, anæsthesia was induced in rats by feeding a dose of 75 mgm. of a solution of 2-AAF which contained no ethanol; for reasons of solubility the solution was administered at a temperature of 43°. Likewise, profound anæsthesia was induced by a single feeding of 2-aminofluorene (150 mgm.) dissolved in sesame oil, 3 ml., containing no ethanol.

Suppression of Mammary Cancer

At 50 days of age, normal female rats were fed a single dose of 3-MC (100 mgm.) and divided into groups. This feeding was the only experimental variable in the control rats which were maintained as virgins, and all developed mammary cancer (Table 2). Indeed, we have shown above that a single feeding of 3-MC (100 mgm.) induced mammary cancer without exception in normal female rats of this age, and that the tumours were demonstrated although not visible to the unaided eye after two weeks. Accordingly, at 65 days of age, the endocrine status of rats in many of the groups was modified drastically by breeding or by administration of steroid hormones.

The expected incidence of mammary cancer was lessened notably in rats bred 15 days after their solitary feeding (Table 2). Moreover, the daily injection of progesterone (4 mgm.) or of œstradiol-17β (10 μgm.) beginning at age 65 days, caused a similar decrease in the rate of incidence. The combined administration of progesterone (4 mgm.) with œstradiol-17β (1 or 10 μgm.) each day for 50 days induced the characteristic gestational type of hyperplasia of the mammary glands, which resembles that found in rats on day 15 of pregnancy, but few mammary cancers ever developed in these rats (Table 2); the neoplasms had been suppressed by the hormonal treatments.

In earlier work[11] the repeated feeding of 3-MC to rats at the onset of pregnancy, or to other animals

Table 2. SUPPRESSION OF MAMMARY CANCER IN PREGNANCY AND IN RATS INJECTED DAILY FOR 50 DAYS BEGINNING 15 DAYS AFTER A SINGLE INTRAGASTRIC INSTILLATION OF 3-MC (100 MGM.)

Steroids injected	No. with cancer	Appearance of palpable tumours (days)			Active centres
		range	median	mean	
None: normal female	10	46–84	56	58·3±11	1·6
None: pregnant	6	30–76	50	48·6±18	2·2
Progesterone (4 mgm.)	8	35–121	46	61·4±30	2·0
Œstradiol-17β (10 μgm.)	7	77–115	97	96·3±13	2·3
Progesterone (4 mgm.) and œstradiol-17β (10 μgm.)	2	42 and 121	—	—	1·0
Progesterone (4 mgm.) and œstradiol-17β (1 μgm.)	3	42–133	45	—	1·0
Progesterone (4 mgm.) and œstradiol-17β (0·01 μgm.)	8	36–120	43	51·9±28	3·2

There were 10 rats in each group and 3-MC (100 mgm.) was administered to every rat at age 50 days. All the animals were virgin females except one group which were bred between age 65 and 68 days; steroids were not administered to these groups. In the others, hormones dissolved in sesame oil (0·2 ml.), were injected intramuscularly 6 days each week for 50 days beginning at 65 days of age. The rats were observed for 150 days.

injected with large quantities of equine gonadotrophin[3], seldom induced mammary cancer. The present experiments demonstrate an effect of a different kind since they show that œstradiol with progesterone, while inciting brisk growth of normal mammary glands, often destroyed the young cancers induced within the previous two weeks by a large dose of 3-MC.

Discussion

Induction of cancer by a solitary feeding of polynuclear carcinogens emphasizes the high importance of the alimentary tract as a portal for their entry and, indeed, of a single meal for the development of mammary cancer in the rat. This method has advantages over multiple feeding in its extreme simplicity and in saving of labour and of rare or costly compounds associated with equal rapidity, and invariability of development of multiple mammary cancers. A stoichiometric relationship exists between the dose of effective carcinogens and the rate of incidence of the tumours, together with their number. The mammary cancers arise under highly restricted conditions, but these are easily fulfilled. All young adult female rats of the Sprague–Dawley strain fed an optimal amount of DMBA developed mammary cancer under the favourable hormonal conditions which prevail in them. Pregnancy, or the administration of progesterone with œstradiol, frequently destroyed many young cancers that were obtaining a foothold, and rats in this class never suffered a recrudescence of their mammary cancers.

Not all the cells of the body were injured by a single moderately large feeding of DMBA and lesions developed selectively. The hæmatopoietic and lymphatic systems were damaged and two types of cancer were induced, while the production of gonadotrophin by the hypophysis, and of phenolic œstrogens by the ovary, were undisturbed.

Not all the cells of the responding animals became malignant after feeding the effective polynuclear hydrocarbons; cancer developed only in the mamma and in special sebaceous glands, while sarcoma was never encountered. All the mammary cancers were adenocarcinomata and squamous carcinoma was not observed in the breast. Yet in companion rats the intramuscular injection of the effective carcinogens evoked sarcoma, locally, in most of them when these carcinogens were injected in muscle. The properties which endow special cells of the body with selective vulnerability to trace amounts of polynuclear hydrocarbons are unknown.

Not all the many millions of cells of the mammary glands became malignant after feeding effective amounts of the carcinogens. The maximal number of mammary cancers which has been observed in this laboratory after a single feeding is 21. A threshold response to the hyperconjugated hydrocarbons is involved in certain cells of the mammary gland in the induction of cancer of the breast by hydrocarbons, since a minimal effective dose gives rise to only one active centre.

Berenblum[12] has postulated that carcinogenesis develops in multiple independent stages. The present experiments demonstrate that mammary cancer, under the stated conditions, arises in a single step.

This work was aided by grants from the American Cancer Society and from the Jane Coffin Childs Memorial Fund for Medical Research.

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TERMINOLOGY IN PLANT- AND SOIL-WATER RELATIONS

A RECENT article[1] under the above title seems unnecessarily complicated and in some respects misleading.

Consider the transfer of water, through a membrane permeable only to water, from a solution α at temperature T, at pressure P^{α}, and having concentration(s) m^{α} of solute(s), to a solution β at temperature T, at pressure P^{β}, and having concentration(s) m^{β} of solute(s). Water will pass spontaneously from solution α to solution β, *or* the two solutions are in equilibrium, *or* water will pass spontaneously from solution β to solution α, according as:

$$\mu_W(T,P^{\alpha},m^{\alpha}) - \mu_W(T,P^{\beta},m^{\beta}) \gtreqless 0 \qquad (1)$$

where $\mu_W(T,P,m)$ is the chemical potential of water in a solution at temperature T, at pressure P, and having concentration(s) m of solute(s). We now identify β with pure water at temperature T and at atmospheric pressure by putting $P^{\beta} = 1$ atm. and $m^{\beta} = 0$, and define a dimensionless number W called the 'water number' of the solution α (whether α is a solution in a beaker, a compressed solution in a turgid cell, soil-water surrounding the roots of a plant, or otherwise) by the relation:

$$W = \{\mu_W(T,P^{\alpha},m^{\alpha}) - \mu_W(T,1\text{ atm.},0)\}/RT \qquad (2)$$

where R is the gas constant. The water number W is then an exact thermodynamic discriminant determining according to its sign whether pure water will flow into (< 0), or out of (> 0), the solution α. Similarly water will flow from a solution with water number W_2 to a solution with water number W_1 if $W_2 > W_1$ and conversely. A solution with a negative water number 'wants' to gain water; a solution with a positive water number 'wants' to lose water.

In order to express the water number in terms of more familiar experimental quantities we begin by rewriting equation (2) in the form:

$$RTW = \{\mu_W(T,P^{\alpha},m^{\alpha}) - \mu_W(T,1\text{ atm.},m^{\alpha})\} + \{\mu_W(T,1\text{ atm.},m^{\alpha}) - \mu_W(T,1\text{ atm.},0)\} \qquad (3)$$

and then evaluate the two terms separately. With only trivial inaccuracy we then obtain the following set of thermodynamically equivalent relations ; which one is used, is entirely a matter of experimental convenience :

$$W = (P^{\alpha} - 1\ \text{atm.})V_{\text{W}}/RT + \ln (p_{\text{W}}/p^{\bullet}_{\text{W}}) \qquad (3a)$$

where V_{W} is the molar volume of water, p_{W} is the partial pressure of water over the solution, and $p^{\bullet}_{\text{W}}$ is the vapour pressure of pure water :

$$W = (P^{\alpha} - 1\ \text{atm.})V_{\text{W}}/RT - k_f \Delta T_f \qquad (3b)$$

where k_f is the cryoscopic constant of water and ΔT_f the lowering of the freezing point (equation 3*b* is, of course, restricted to 0° C.) :

$$W = (P^{\alpha} - 1\ \text{atm.})V_{\text{W}}/RT - k_{\text{b}} \Delta T_{\text{b}} \qquad (3c)$$

where k_{b} is the ebullioscopic constant of water and ΔT_{b} the elevation of the boiling point (equation 3*c* is, of course, restricted to 100° C.), and :

$$W = (P^{\alpha} - 1\ \text{atm.})V_{\text{W}}/RT - \Pi V_{\text{W}}/RT \qquad (3d)$$

where Π is the osmotic pressure of the solution.

Of the four methods of measuring the second term in equations (3) the third has in practice been used scarcely at all, while the first and second have been used much more often, and usually with much higher accuracy, than the fourth. Results obtained by any of these four methods are often expressed as osmotic coefficients φ in terms of which we have the alternative relation :

$$W = (P^{\alpha} - 1\ \text{atm.})V_{\text{W}}/RT - M_{\text{W}}\varphi m^{\alpha} \qquad (3e)$$

where M_{W} is the molar mass of water and m^{α} is the sum of the molalities of all the solutes in the solution. Tables of φ for solutions of common solutes (including sucrose) are available[2].

Equations (3*a*) to (3*e*) are thermodynamically equivalent. Equation (3*d*) can be rearranged in terms of quantities with the dimensions of pressure as follows :

$$-RTW/V_{\text{W}} = \Pi - (P^{\alpha} - 1\ \text{atm.})$$

or in terms of a few of the names commonly used by botanists: suction pressure (or diffusion pressure deficit) = osmotic pressure − wall pressure (or turgor pressure). In my opinion the apparent simplicity of this formulation, together with the traditional preoccupation of botanists with osmotic pressures to the neglect of more important ways of measuring essentially the same quantity, has led to a great deal of confusion, misunderstanding and pointless argument (as well as to the corruption of mechanical terms such as 'pressure', 'force', 'stress' and 'potential'). In particular, while the wall pressure is the actual excess pressure over 1 atm. in a more or less turgid cell, neither the suction pressure nor the osmotic pressure is an actual pressure in any part of a plant during its approach to equilibrium with its surroundings.

The definition of water number can easily be extended to systems where variables other than pressure and concentration are important. For example, if the difference of heights $h^{\alpha} - h^{\beta}$ is important (as in a tall tree) the gravitational term $M_{\text{W}}g(h^{\alpha} - h^{\beta})$ must be added to the left-hand side of equation (1).

Water numbers and indeed any thermodynamic treatment can, however, only be applied to a given system (for example, soil + plant + air) when the whole of that system is at one and the same temperature (cf. par. 6 of ref. 1, where the contrary is stated).

The application of water numbers to practical botany will be described more fully elsewhere.

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DR. McGLASHAN's concept of 'water numbers' is an alternative step in the direction suggested by the delegates to the Unesco–Spain symposium on plant water relations in arid regions[1]. It, too, proposes a single term to replace the larger number at present in use and has simply and clearly partitioned this quantity into measurable components.

However, as a substitute for the proposals it does not encompass the identifiable components that may contribute to the 'water potential'. The symposium delegates felt that the additional factors which were recognizable should be given consistent names, whereas the use of 'water numbers' provides a name for the total potential only.

In general terms, the symposium delegates were keen to develop a terminology which would be more comprehensive than the existing plant water systems. To this extent, pressure, matrix and osmotic components were introduced, recognizing that, for example, the matrix term might not be necessary to evaluate the total water potential in plants or the osmotic component necessary to evaluate the total components in soils. It was also envisaged that other component potentials due to external force fields, such as gravity and electricity, could be included if necessary or desirable.

Dr. McGlashan's proposal of 'water numbers' is identical with our 'water potential' (that is, the difference in the Gibbs function for the single component, water, in a multiple component system and the same component in the reference state). He does not provide for naming the several factors that contribute to the overall water potential as we have done. His equations 3*a*–3*e* are equivalent relations expressed in terms of various methods that might be used to evaluate the chemical potential under standard laboratory conditions. These equations all represent standard methods for evaluating the chemical potential of water in pure systems.

Methods based on evaluation of the freezing or boiling point of water are subject to errors of unknown magnitude since they assume that the entropy and enthalpy of the water in system behave the same as those of pure free water. This is known to be incorrect at least for soil water[2], so that the values determined by these methods can be applied, without error, only at the freezing and boiling temperatures.

The application of the classical Gibbs function (and hence the water number) to systems that are not at constant temperature is not possible because flow of heat occurs whenever there are temperature differences. This upsets the equilibrium conditions, thus invalidating the equilibrium thermodynamics. This is also true in any system in which any kind of flow is taking place. It is still possible, however, to use thermodynamic concepts and terminology for non-equilibrium systems, if the change in entropy of the system is considered[3]. The terminology proposed by the delegates to the Unesco–Spain symposium is adaptable to applications of thermodynamics to irreversible processes, hence temperature differences may be included in the terminology. Likewise, the water potential (or water number) determined on a system in equilibrium at one temperature will be different from the water potential of the entire system in equilibrium at another temperature, therefore it is necessary to consider a temperature (and hence an entropy) contribution to the water potential.

It might be mentioned that at the request of the symposium delegates, we also circulated the proposals to a large number of scientists known to be interested in the subject of terminology in plant–soil–water systems. The range of response varied from complete rejection to complete acceptance, with most correspondents being in favour of general acceptance with some modifications. In general, most scientists concerned with either soil- or plant–water relations alone considered that either the existing terminologies were satisfactory, or that the new proposals compromised the existing ones to a degree that was not of benefit. The majority of scientists concerned with the interacting plant–soil–water system were, on the other hand, keen to have a more unified form of expression. These varied opinions have been summarized, and the original proposals modified to include as many of the comments as possible. The resultant document will be published shortly by Unesco, in the Proceedings of the Madrid symposium.

The original proposals were also discussed at the recent congress of the International Soil Science Society at Madison, Wisconsin. Here again a wide range of response was encountered, and Commission I (Soil Physics) of the International Society of Soil Science has appointed a committee to investigate the subject of soil water terminology.

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RADIAL PARTICLE DISPLACEMENTS IN POISEUILLE FLOW OF SUSPENSIONS

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WE have observed that macroscopic spherical particles collect into a thin, annular region when an initially uniform dilute suspension is passed in laminar flow through a straight tube. The development of the effect is proportional to the length l of the tube, to the mean velocity V of flow and to the fourth power of the ratio of particle radius a to tube radius R.

Our equipment consisted of the flow apparatus, an optical scanning device and an electronic counting circuit. Spheres of polymethyl methacrylate, in various uniform fractions of diameters between 0·8 and 1·6 mm., were suspended in a liquid of the same density $\rho = 1{\cdot}18$ gm./c.c. and of high viscosity $\eta = 400$ cp.). The flow apparatus consisted of a vertical tube of variable length, diameter 11·6 mm., terminating in two containers with the necessary stirrers and pressure-head regulators. At a selected cross-section of the tube, two narrow mutally perpendicular light rays could be made to traverse it without distortion and thus to determine a precisely selected small region at their crossing. Two photocells and a coincidence circuit permitted the computation of the actual number of particles which passed the point of intersection. From this number the average concentration of the suspension, as a function of the distance from the axis of the tube, could be calculated by dividing by the local velocity of flow.

The results are symmetrical about the axis, and are independent of the shape of the mouth of the tube. At very low velocities of flow, or at small distances from the input end, the particle count follows a parabolic distribution, showing that the concentration is constant over the entire cross-section (curve 1, Fig. 1).

At cross-sections farther down the tube, distributions of the type shown in curves 2 and 3, Fig. 1, are observed. In terms of particle concentrations (assuming the same parabolic velocity distribution as at the mouth) the results conform to the 'tubular pinch' effect illustrated in Fig. 2.

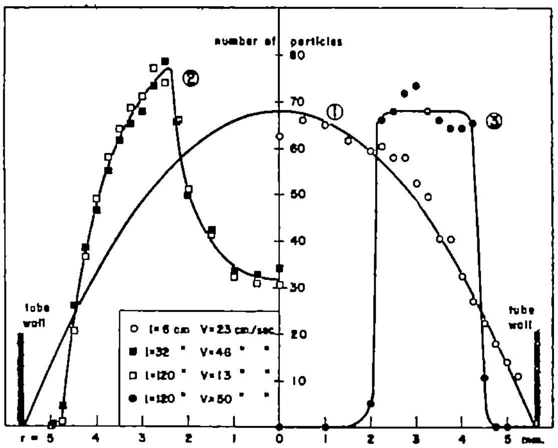

Fig. 1. Number of particle passages as a function of radial position in tube for a given total throughput (uncorrected for finite scanning cross-section). Particle radius 0·8 mm., initial concentration 1 part./cm.^{3}

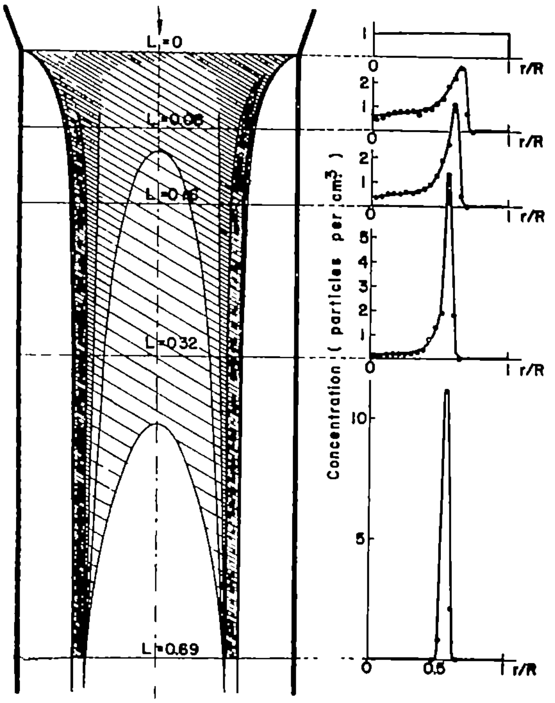

Fig. 2. The 'tubular pinch' effect: particle concentration as a function of radial and longitudinal position in tube. Initial concentration 1 part./cm.³. Concentrations are indicated by shading; closest shading (more than 2 part./cm.³); next (1–2 part./cm.³); next (0 5–1 part./cm.³); lightest shading (0–0 5 part./cm.³)

The results in Fig. 2 are plotted in terms of a reduced tube radius (r/R) and tube length:

$$L = (a/R)(av\rho/\eta)(l/R)$$

where $v = (a/R)^2V$, and $(av\rho/\eta)$ can be defined as a relative particle Reynolds number[1]. All our results can be superimposed when plotted in these coordinates, that is, the same particle distributions are reached at corresponding positions L in the tube. The dependence of L on the relative particle Reynolds number, or more explicitly on the product Vl, can be shown to imply a radial particle velocity proportional to V^2. The force responsible for the effect thus originates in the inertia of the fluid.

Particle re-arrangements in parabolic flow have been suspected in the past, and certain anomalous flow-behaviour of suspensions has been explained in this way. Causes other than inertia forces have, however, been made primarily responsible as no direct observations of particle movements were made. Deviations from the Einstein viscosity equation (sigma effect) were explained by wall effects[2] and the criterion of minimum energy dissipation[3].

Some direct observations of particles concentrating in the central region of the tube[4] have been reported, but the relevant experiments seem to be of low precision or may have been complicated by electrostatic and other phenomena, as, for example, in the case[5] often quoted of red cells moving into the axis of blood capillaries.

On the theoretical side, the effects of fluid inertia have almost always been neglected in view of the difficulty of solving the complete Navier-Stokes equations of motion. Cases such as ours are thus explicitly excluded from available analyses of tube flow. The only way in which quadratic terms have been allowed for in a few cases[6,7] is through the inclusion by postulation of a transverse force which, as in the 'Magnus effect', arises from the combination of a rotatory and a translatory motion relative to the undisturbed flow of the fluid. It can be shown[8] by solving the linearized equations of motion that a spherical particle carried along by an unbounded parabolic field of flow is subjected to a couple and to a longitudinal force which give rise to a rotation and a translation, each proportional to the mean velocity of flow. Adopting the formalism of the Magnus effect, one can substitute these velocities in the Kutta–Jukowsky formula and obtain a force:

$$f = -k(a^5V^2r\rho/R^4)$$

and a radial velocity $\dot{r}$ corresponding to this force:

$$\dot{r} = -(k/6\pi)(a/R)^4(V^2\rho r/\eta)$$

proportional to V^2, as observed, but directed inwards only. In our case the radial force acting on the spheres is centripetal at the wall but centrifugal at the centre. A stationary distribution is thus reached, and the value of (r/R) where the particles concentrate seems to be independent of all other variables.

It is worth noting that if we assume for the dimensionless coefficient k the value suggested by Tollert[7] or that calculated by Saffman[6], agreement in order of magnitude with the observed centripetal removal of a particle from the wall is obtained.

This approach does not explain the strange dependence of the force on the distance from the centre with its reversal of sign. The curvature of the field of flow is patently insufficient to account for this behaviour. It is in the presence of a rigid wall at $r = R$ that the explanation must be sought. It should be noted that symmetry requirements are satisfied, as a force proportional to r, and thus, vanishing at the centre, is consistent with the observed removal of particles in the vicinity of the tube axis. On the other hand, the criterion of minimum energy dissipation according to which one would expect the particles to congregate in the centre is contravened.

The results exemplified by Fig. 1 are independent of the overall particle concentration up to the largest volume fraction (0·4 per cent) used by us, and a linear superposition of the effect is observed when particles of two different sizes are examined simultaneously. The points represent averages involving at least 20 separate experiments and the scatter shown in the diagrams is representative of the precision obtained.

The effect may be important in explaining certain anomalous flow behaviour of suspensions and may find application in the fractionation of particles of different size.

To the best of our knowledge the centrifugal effect has not previously been observed nor are any theoretical predictions extant. The investigation is being pursued, and a full report will be published elsewhere.

Acknowledgements are due to Prof. A. Katchalsky for many valuable discussions and to Cartiera Vita Mayer and Co., Milan, for enabling one of us (G. Segré) to carry out this investigation at the Weizmann Institute.

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LETTERS TO THE EDITORS

PHYSICS

Glass Formation in Solutions of Hydrogen Peroxide–Water and Deuterium Peroxide–Heavy Water

We have found that if a hydrogen peroxide–water solution, of suitable concentration, is put into a tube and frozen extremely quickly by plunging the tube into a Dewar flask containing liquid oxygen (90° K.), a transparent colourless solid is formed. This solid has the properties of a glass, in that it is both transparent and amorphous. A glass is also formed if the hydrogen peroxide solution is allowed to fall as drops on to liquid oxygen.

The hydrogen peroxide used was high-test unstabilized hydrogen peroxide from Laporte Chemicals, Ltd. The various concentrations were made, either by mixing the standard concentrations supplied by Laporte or by dilution of approximately 95 per cent w/w hydrogen peroxide with laboratory-distilled water. No attempt was made to de-gas the solutions. When necessary, the hydrogen peroxide concentrations were checked by potassium permanganate titrations, and these were accurate to 1 per cent.

The most convenient method of testing whether a given concentration forms a glass or not was found to be that described by Livingston and Zeldes[1]. The liquid solution at room temperature was allowed to fall as drops, of 2–5 mm. diameter, on to the surface of liquid oxygen. Initially, the drop floated on the surface of the liquid and froze to form a sphere. For concentrations 45·5–87 per cent w/w, perfectly transparent glass spheres were formed; for all other concentrations, opaque white spheres were formed, and these were presumably polycrystalline.

A glass is again obtained when hydrogen peroxide, of 45·5–87 per cent w/w, is put into a glass tube, on to an aluminium plate or between glass plates and then frozen in liquid oxygen. The glass now, however, has very marked cracks. The formation of cracks is presumably due to thermal contraction. No cracks were found in the case of the spheres, since there are no constraining walls on the sample. For all samples the glass is completely stable and no transformations were found on storing at 90° K. for two days.

The boundary at 45·5 per cent w/w between the concentrations which produce polycrystalline material and those which produce a glass is very sharp and corresponds to the eutectic point (Banfield, R. H., private communication) at 45·3 per cent w/w. The boundary at 87 per cent w/w is far less marked and can vary by ± 5 per cent w/w; this concentration is well above the second eutectic point (Banfield, R. H., private communication) of 60·5 per cent w/w, but it coincides, however, with a well-marked change in the slope of the melting point/concentration curve for hydrogen peroxide–water solutions as determined by Giguère and Maass[2] and by Kubaschewski and Weber[3] from measurements on quickly frozen (non-equilibrium) solutions.

Cooper and Watkinson[4] made measurements on the supercooling of hydrogen peroxide–water solutions, using slow cooling. They found supercooling for concentrations between 50 per cent w/w and 97 per cent w/w (the highest concentration used); at 86 per cent w/w they recorded the lowest freezing temperature for supercooled liquid. Cooper and Watkinson also reported the formation of a glass at 86 per cent w/w, when the sample at 194·5° K. was transferred to liquid nitrogen (77° K.). Bolz, Mauer and Peiser[5] performed X-ray diffraction studies on 90–95 per cent w/w hydrogen peroxide (Mauer, F. A., private communication) condensed from the vapour at 77° K. For temperatures below 92° K. they found an amorphous glass giving very broad (8°) diffraction lines.

We have also studied the effect of warming the glass samples. The solution to be tested was put into a small test-tube holding about 5 ml. of solution. A thermocouple was put into the centre of the solution and the whole assembly plunged into liquid oxygen; a glass was formed with some cracks. The leads of the thermocouple were connected to a potentiometric pen recorder of 1 sec. response-time. The accuracy of temperature measurement was ± 2° K. The tube was removed from the liquid oxygen and was placed in the air at room temperature. A typical warming curve is shown in Fig. 1 for 61·0 per cent w/w hydrogen peroxide. The quick rise of temperature *AB* is associated with the appearance of opacity in the sample which spreads along the tube, and this is presumably due to exothermic crystallization of the solution. Several measurements were made on 61·0 per cent w/w samples, and for these it was found that crystallization occurred at 165 ± 6° K. Similar behaviour was found by Bolz, Mauer and Peiser[5], the crystallization for 90–95 per cent w/w hydrogen peroxide, however, commencing at 92° K. The region over which the temperature remains constant, *CD* in Fig. 1, is due to melting of the crystals and was found to be at 217 ± 2° K. This is in agreement

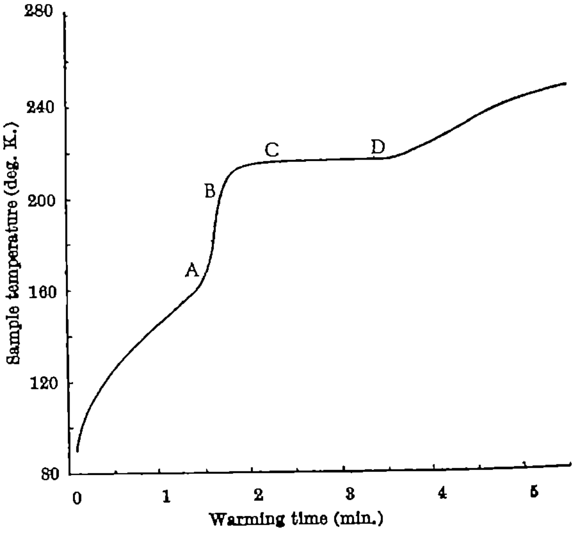

Fig. 1. Warming curve for 61·0 per cent w/w hydrogen peroxide

with measurements of the melting points by Giguère and Maass[2], and by Kubaschewski and Weber[3]. We have tested two other concentrations, 45·5 per cent w/w and 80 per cent w/w and these showed a similar behaviour, crystallization taking place at 141 ± 10° K. and 157 ± 10° K. respectively. The melting points of these two concentrations were found to be 222 ± 5° K. and 215 ± 5° K., in agreement with published measurements[2,3]. In all the glass samples the cracks annealed out between 130 and 155° K., that is, before the crystallization took place.

Tests of glass formation were also made using deuterium peroxide, obtained from Laval University. The concentration used was 80 per cent w/w, with 99 per cent deuterium. It was found that a stable glass was produced as above, both in spheres and in a glass tube. Giguère and Secco[6] have also reported the formation of glasses in deuterium peroxide, but on storing in liquid air for 10 min. they found a spontaneous conversion to crystallization. We have not found this effect and cannot explain the difference between the two results.

These observations were obtained during experiments involving ultra-violet irradiation of hydrogen peroxide–water solutions at 90° K. for electron spin resonance spectroscopy[7].

The potassium permanganate titrations were performed by Mr. J. M. Richards of this Department. We wish to acknowledge helpful discussions with Mr. R. H. Banfield of Laporte Chemicals, Ltd., and, in particular, for his suggestion of an examination of the eutectic mixtures. The hydrogen peroxide for these measurements was kindly supplied by Laporte Chemicals, Ltd. The deuterium peroxide was given to us by Prof. P. A. Giguère of Laval University, Quebec. This work was supported by the British Empire Cancer Campaign, for the assistance of which we express grateful thanks.

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A Spectrometer for the Far Infra-red

A SPECTROMETER based on a Fresnel zone plate as the focusing and dispersing element has been constructed for use with infra-red radiation between 35μ and 80μ.

The instrument is intrinsically simple. A heated nichrome coil and a Fresnel zone plate are mounted on opposite ends of a 4-m. *I*-beam so that the zone plate produces a series of monochromatic images of the source disposed along the axis of the instrument. A bolometer detector mounted on a carriage moves along a straight track attached to the *I*-beam so as to scan the spectrum. A light chopper in front of the source uses alternating open holes and potassium bromide windows to eliminate virtually all but first-order spectra from the signal. The sample cell is located in the light path close to the source.

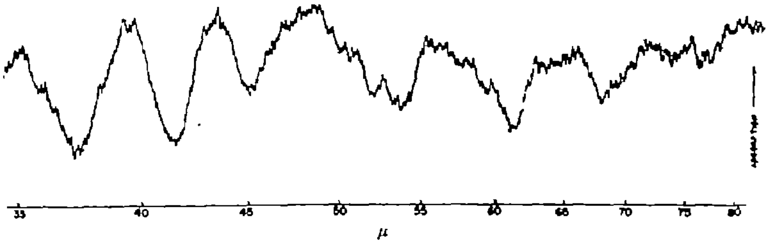

Fig. 1

The zone plate is of the reflecting type, containing 234 half-wave Fresnel zones. The surfaces of the even-numbered zones are raised 12·5μ above those of the odd-numbered zones to give a maximum optical efficiency at 50μ. The zone radii are calculated to make the system free from spherical aberration at 68μ and to give the zone plate a focal length of 73·5 cm. at that wave-length.

The zone plate focuses a monochromatic image of the source directly on the bolometer element with no intervening collimating mirrors, or movable slits, as in a grating spectrometer. The simplicity of this design leads to ease of construction and good light-gathering power. Fig. 1 shows the absorption spectrum of atmospheric water vapour.

Patents have been applied for.

We wish to thank the Trans Mountain Oil Pipe Line Co. and its president, Dr. D. M. Morrison, for a grant which made possible the construction of this instrument.

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Elimination of a Glow Discharge in an Induction-heated Vacuum Furnace

RECENTLY, J. P. Burden[1] discussed the elimination of a glow discharge in induction-heated vacuum furnaces. As Burden indicated, the glow discharge is troublesome because it robs energy. In addition, in vacuum analysis systems, the discharge is often intolerable because of the consequent evolution of gases from glass furnace parts.

For the past three years, an almost perfect method of eliminating the glow discharge has been employed in this Laboratory. It was found that a small horseshoe permanent magnet placed near the glass furnace wall prevents the initiation of a discharge, or immediately quenches a discharge once started. The exact position of the magnet varies slightly, and must be determined empirically, but is about one centimetre or less from the furnace wall. The system to which the magnet 'trick' has been applied is a conventional vacuum fusion analysis apparatus with an all-glass, air-cooled furnace[2] inductively heated by a 2·5-kW., 500-kc. oscillator. The idea of using a magnet in the manner indicated was suggested by the common application of a permanent magnet as an 'ion trap' on television picture tubes.

Strangely enough, the use of the magnet has twice been observed to have the opposite of the desired effect. That is, a glow discharge was initiated when the magnet was brought near the furnace, and extinguished on removal of the magnet.

No attempt will here be made to suggest how the magnet succeeds (or rarely, does not succeed) in eliminating a glow discharge. Suffice it to say that the success has been just short of perfection, and the more gratifying because it was attained at little expense and much convenience.

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Visible Twist on Sodium Chloride Whiskers

IT is well known from Eshelby's[1] work that a whisker containing an axial screw dislocation will have the form of a twisted rod. Sodium chloride whiskers have been grown in which such a twist was quite visible to the naked eye.

The whiskers were prepared by a modification of Evans's method[2]. A small sodium chloride crystal grown from the melt was placed in a solution of sodium chloride, containing a small amount of polyvinyl alcohol. The whiskers grew on this seed-crystal. The mean length of the whiskers was 150 mm., their thickness 1–30μ and they were rectangular in cross-section. Some of the whiskers, mainly the thinner ones, were spiral. The twisted form was easily visible to the naked eye: with suitable light brighter and darker spots became visible along the lengths of whiskers, because due to their twisted form only some parts of them reflected the light. The distances between light spots on most of the crystals were 1–40 mm. and they were uniformly distributed along the length of the whiskers. It is suggested that this is probably a twist described by Eshelby. He gives the formula for the twist per unit length as follows:

$$\text{twist } \alpha = k \times \frac{\text{Burgers vector } b}{\text{area of whisker cross-section } A}$$

where k is a constant depending on the shape of the cross-section. The Burgers vector of the screw dislocation calculated with this formula results in values of 60–240 Å. These are the results of measurements carried out on twelve whiskers. Webb[3] obtained similar results among others by the X-ray diffraction method.

In our opinion, polyvinyl alcohol plays little part in the production of whiskers, because Gyulai[4] also found spiral whiskers in aqueous solution of potassium bromide containing no polyvinyl alcohol. The whiskers were tested by twisting. Fig. 1*a* shows a spiral whisker having eleven light-spots. The same whisker is shown in Fig. 1*b*, twisted outwards through 360°. It can be seen that the number of light-spots has diminished by four. Twisting further through 360°, the number of light-spots diminished to three (Fig. 1*c*). Twisting the whisker through 990°, it became quite smooth, and light-spots were no longer visible (Fig. 1*d*). If one end is cut the whisker twists back again. After this twisting-out the twisting-back phenomenon was observed in each test. In most cases the whisker was almost completely twisted back. It appears that this phenomenon disagrees with Eshelby's suggestion, according to which after a certain twisting the dislocation leaves the whisker. There may be several explanations.

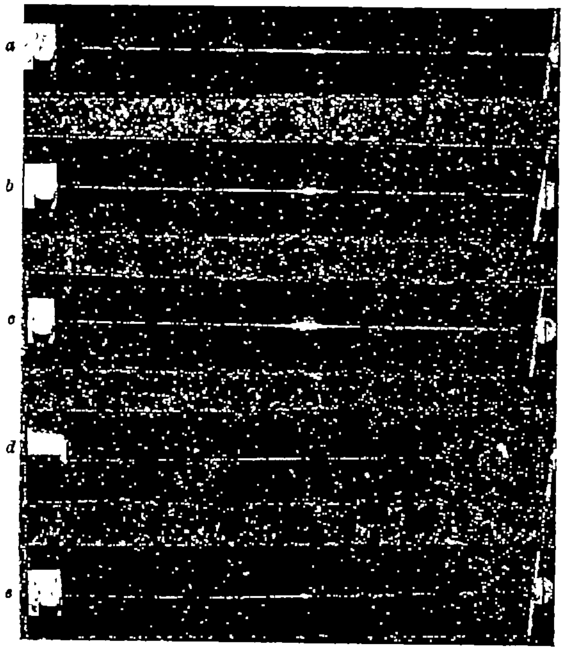

Fig. 1. *a*, Naturally twisted sodium chloride whisker (original length); *b*, the same whisker twisted through 360°; *c*, the same whisker twisted through 720°; *d*, the same whisker twisted through 990°; *e*, the same whisker, one of its ends cut off after twisting, and the whisker quite twisted back

Further investigations on whiskers are in progress. A detailed paper will be published in *Acta Physica Hungarica*.

We wish to thank Prof. Z. Gyulai for valuable discussion and interest.

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Weissenberg Effect in the Thick White of the Hen's Egg

WHEN a vertical rod is rotated in certain liquids, the liquid climbs up the rod. This phenomenon was apparently first observed by Weissenberg and Russell[1], and in 1949 Reiner, Scott Blair and Hawley[2] suggested that it be described as the 'Weissenberg effect'.

Its theory is not clear and has been discussed by Garner and Nissan[3], Rivlin[4], Reiner *et al.*[3] and Roberts[5]. The effect occurs only rarely and has been observed in highly viscous solutions of rubber in benzene[3], certain soaps in hydrocarbon solvents[3], over-aged sweetened condensed milk[2] and certain types of dilatant honey[6].

During an investigation into the rheological behaviour of cake batters it was found that most of them exhibited the Weissenberg effect to a greater or lesser extent dependent on their egg content. When the separate constituents of egg were tested it was apparent that the more viscous component of the albumen, the so-called thick white[7], sometimes also referred to as the middle dense layer or albuminous sac[8], exhibited the Weissenberg effect most markedly. This material was readily isolated by a method virtually identical with that of Brooks and Hale[7]. The eggs were broken individually on a clean B.S. 14 mesh sieve which had been wetted with water and the outer thin white was allowed to drain off. After tilting the sieve gently to and fro for 1 min., the white around the yolk was cut and the yolk removed by hand. After draining off the inner thin white, the thick white remained on the sieve and could be used for the test.

The thick white of the hen's egg thus provides an easily accessible material by means of which the Weissenberg effect may be demonstrated.

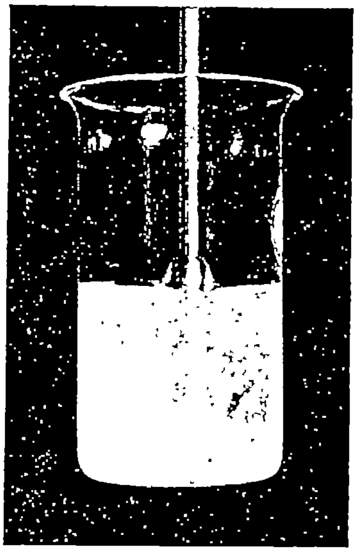

Fig. 1

I wish to thank Mr. A. W. Hartley for Fig. 1, which shows the Weissenberg effect in the thick white.

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GEOPHYSICS

Lunar Tide in the F_2 Layer of the Ionosphere near the Geomagnetic Equator

It is well known that the maximum in the lunar tide variation of f_0F_2 occurs at about 10 lunar hours at moderate geomagnetic latitudes and at about 04 lunar hours near the geomagnetic equator[1,2]. Recent analyses of the lunar variations at Singapore, Ibadan and Bombay have indicated non-agreement in the phases of the variation at stations having the same geomagnetic latitudes.

In this communication the analysis of lunar variations of f_0F_2 at Leopoldville for the period of three years from November 1953 to October 1956 is presented. From the published hourly values of f_0F_2, daily averages of three-hourly values between 11 hr. to 13 hr. (15° E.M.T.) are computed and are referred to as midday values. The 27-day running averages of these values are computed and are subtracted from the individual day values for the central day of the respective 27-day period to obtain the deviation of f_0F_2 for that day. The deviations of f_0F_2 for days having the same phase (μ) of the Moon are then grouped together, and thus 24 such groups are obtained for the different phases of the Moon. The average deviations of f_0F_2 for each phase are computed separately for the months of southern solstice (November, December, January and February), for equinoxes (March, April, September and October), for the northern solstice (May, June, July and August) as well as for the whole year. To avoid the effects of

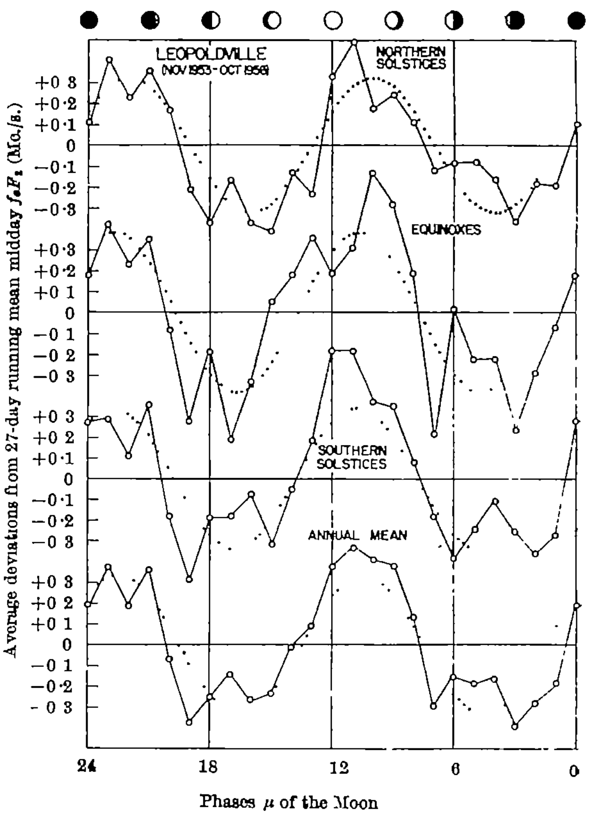

Fig. 1

Table 1. AMPLITUDE P_1 AND PHASE t_1 OF THE LUNAR VARIATION OF NOON f_0F_2 AT LOW LATITUDE STATIONS

Station	Geomagnetic latitude	Magnetic latitude	P_1 (Mc./s.)	t_1 lunar hour	Reference
Huancayo	0·6° S.	0°	0·45	4·0	1
Leyte	0·2° S.	3° N.	0·53	4·2	1
Tiruchy	1° N.	2·5° N.	0·18	2·8	1
Christmas Islands	2·1° N.	2·5° N.	0·33	4·7	1
Madras	3·1° N.	5·5° N.	0·14	3·8	2
Ibadan	10·4° N.	2·5° S.	0·38	3·3	4
Singapore	10·0° S.	8° S.	(0·30)	(4·0)	3
Leopoldville	3·1° S.	19° S.	0·33	10·5	this communication
Bombay	9·8° N.	12·5° N	0·24	10·0	2
Ahmedabad	13·6° N.	18·6° N.	0·35	10·2	2
Trinidad	22° N.	28·5° N.	0·31	9·2	1
Maui	20·9° N.	21·5° N.	0·36	9·1	1

the magnetic disturbances, the days with A_p value greater than 50 were excluded in averaging. Further, to reduce the scatter, the deviations exceeding more than 20 per cent of the corresponding 27-day mean value were also excluded. These eliminated only less than 9 per cent of the total number of available values. The result of the analysis is shown in Fig. 1. It may be mentioned that from one day to the next μ decreases by 0·81272, and the lunar time corresponding to a phase μ is given by $\mu \pm 12$ (lunar hours).

In Table 1 are compared the amplitudes and phases of the lunar variations of f_0F_2 at Leopoldville and at a number of low-latitude stations in the world. It is seen that the lunar variation at Leopoldville (geom. lat. $\Phi = 3{\cdot}1°$ S.) is completely out of phase with that at Madras ($\Phi = 3{\cdot}1°$ N.) or at any other equatorial station. The phase of the lunar variation at Leopoldville (magn. lat. = 19° S.) is very nearly the same as that at Ahmedabad (magn. lat. = 19° N.) having the same magnetic latitude. This suggests that the stations having the same magnetic latitude rather than the same geomagnetic latitude experience similar variation of f_0F_2. The equatorial type of lunar variation at Ibadan and the middle latitude type of variation at Bombay in spite of the geomagnetic latitudes of both stations being about 10° can be understood if one considers that the magnetic latitudes of the stations are about 3° S. and 13° N. respectively. The reversal of phase of the lunar tide in f_0F_2 takes place around magnetic latitude 10° or at magnetic dip 20°.

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Spread-*F* and the Latitude Variation of Occurrence of Whistlers

RECENT work of whistling atmospherics has suggested that the incidence of the phenomenon is dependent on latitude. Crouchley[1], using data from Australian and Japanese stations, demonstrated that whistler activity reaches a maximum at about 45° geomagnetic latitude, while the Stanford group[2] report a maximum of occurrence at 50° geomagnetic latitude. In both these reports a marked decrease in activity at lower and higher latitudes is noted. The decreasing incidence at higher latitudes has been attributed[3] to decreasing thunderstorm activity, and rapidly increasing path-length and absorption with increasing latitude. However, the fall-off in activity at low latitudes cannot be so explained. Smith, Helliwell and Yabroff[3] have suggested an explanation of this low-latitude fall-off in terms of whistler propagation within field-aligned ducts. They showed that the necessary ionization enhancement within the ducts for *F*-layer heights falls off markedly with increasing latitude and argue, therefore, that this suggests that whistler occurrence should increase with latitude, since large enhancements would be expected to occur less frequently than small enhancements. It is the purpose of this communication to examine this point in more detail.

The frequency-spreading component of spread-*F* has been interpreted[4,5] as being due to the availability of a range of values of $N_{\max.}$ at the maximum of the F_2 layer; this range of values is thought to correspond to a system of irregularities each involving an enhancement (or a deficiency) of electron density relative to the background ionization. For any particular occurrence of frequency-spreading it is possible, in terms of this hypothesis and assuming critical reflexion conditions to apply, to calculate the ionization enhancement ($\Delta N/N$) required. It is now suggested that these postulated irregularities in the F_2-layer are, in fact, the lower extremities of the whistler ducts. If this is the case, then estimates of the electron density enhancements obtained from frequency-spreading information should support the contention of Smith *et al.* outlined above.

In work to be published elsewhere I have been able to fix limits to the range of values of ΔN observed at seven stations spread in geomagnetic latitude. Combining this information for a typical winter month with the monthly median critical frequency, it has been possible to produce, as shown in Fig. 1, a diagrammatic representation (shaded strip) of how the range of values of $\Delta N/N$ varies with geomagnetic latitude. (The position of that portion of the shaded strip bounded by dashed lines is less certain than

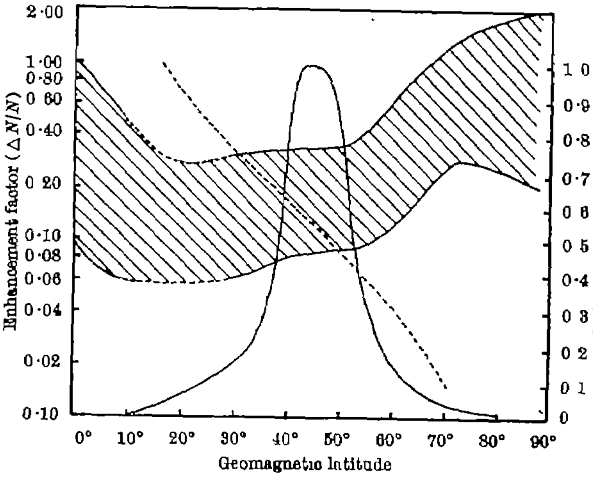

Fig. 1. Observed irregularity of electron density enhancement factors (shaded area) compared with the minimum required for whistler duct propagation (dashed curve) and with whistler occurrence data (full-line curve) for a range of geomagnetic latitudes

elsewhere because of the low incidence of frequency spreading at these latitudes.) Also shown in Fig. 1 is the graph of minimum $\Delta N/N$ against geomagnetic latitude (dashed curve) due to Smith *et al.* and Crouchley's latitude distribution of whistler occurrence. (Since whistler activity is most pronounced in the winter months the latitude distribution of whistler activity is largely dependent upon winter conditions.) It will be noted that for latitudes less than about 30° the observed range of $\Delta N/N$ involves values considerably less than the values required by Smith *et al.* and that this corresponds to the region of low whistler occurrence. At 32° the largest observed value of $\Delta N/N$ corresponds to the minimum required for whistler propagation, while above 50° all the observed values of $\Delta N/N$ are of sufficient magnitude to support whistler propagation. The 30–50° latitude region corresponds to that in which the whistler activity increases. It seems, therefore, that the explanation of the latitude variation of whistler activity based on propagation within field-aligned ducts is consistent with ideas at present being used to interpret the frequency-spreading component of spread-*F*.

I am indebted to Mr. J. Crouchley and Dr. J. A. Thomas for their interest and helpful discussion.

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METALLURGY

Micropitting of Titanium during Anodic Oxidation in Formic Acid

The anodic oxidation of titanium in the majority of electrolytes (sulphuric, phosphoric, boric acid, etc.) results in the formation of a thin homogeneous dielectric oxide film of titanium dioxide (amorphous or crystalline). At constant current the potential increases with increasing film thickness, and, if the potential is then maintained constant, the ionic current decreases rapidly and the current becomes almost entirely electronic. In these circumstances, providing film breakdown does not occur, film growth practically ceases and oxygen evolution becomes the predominant electrode process.

In chloride electrolytes, however, film growth proceeds up to a potential of ~ 12–14 V., and if the potential is increased above this value film breakdown occurs, and titanium ions pass into the electrolyte, and are then precipitated as titanium dioxide. Corrosion of the titanium is confined to a relatively few, widely dispersed, small areas of the metal surface with the consequent formation of gross pits while the rest of the surface, which is coated with the initially formed anodic oxide, remains passive.

During a study of the anodic oxidation of titanium in various electrolytes it has been found that formic acid (and formates) gives rise to a phenomenon which does not appear to have been reported previously. Within a certain range of temperature and concentration of formic acid (Fig. 1, zone *C*) film formation, at constant current, proceeds up to a voltage of ~ 60 V., but as the potential increases above this value, very fine pits are formed uniformly over the metal surface (micropitting) and simultaneously titanium oxide is precipitated from the electrolyte. The potential slowly increases to ~ 120 V., indicating film growth, and then remains practically constant, and if anodizing is continued the pits are propagated into the metal to a considerable depth (Fig. 2). A microscopical examination of the metal surface during the initial stages of micropitting has revealed that different areas on the metal surface have different interference colours showing that the thickness of the surface oxide is not uniform.

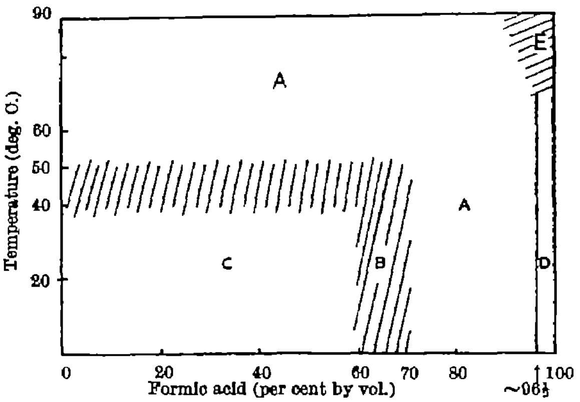

Fig. 1. Effect of concentration and temperature on the anodic behaviour of titanium in formic acid. *A*, Homogeneous dielectric oxide films; *B*, transition zone where homogeneous films show evidence of micropitting; *C*, micropitting; *D*, thick yellow non-adherent oxide films; *E*, transition zone between *A* and *D*

If, however, the temperature of the electrolyte is greater than ~ 50° C. or if the concentration is in the range of ~ 70–96 per cent by vol. formic acid (Fig. 1, zone *A*) micropitting does not occur and a homogeneous dielectric oxide film is formed. It should be observed that at 25° C., at a concentration of ~ 96 per cent formic acid, film formation can proceed to 400 V. without film breakdown, whereas in boric acid the maximum formation voltage is only ~ 250 V.

In region *D*, at very high concentrations of formic acid (~ 96·5 per cent to concentrated), a thick

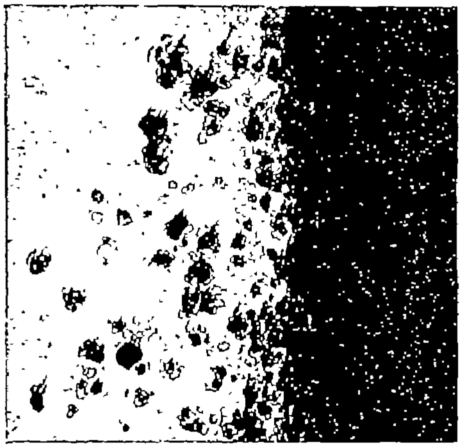

Fig. 2. Section of a micropitted specimen produced using 1 per cent by vol. formic acid, 25° C., 5 m.amp./cm.^{2} for 4 hr. (× *c*. 66). The section has been cut at 5° to the surface to exaggerate depth of penetration of pits. Depth of penetration, $4 \cdot 5 \times 10^{-3}$ cm.

dark-yellow, non-adherent oxide forms on the metal surface.

A consideration of data[1] on the rate of corrosion in different concentrations of formic acid at 35° C. reveals that zone *C* corresponds to the range of concentration where the corrosion-rate is high and region *A* where the corrosion-rate is at a minimum.

It is apparent that the mechanism of micropitting is highly complex, as the electrode processes which occur involve the anodic oxidation of titanium, localized corrosion of titanium, oxidation of formic acid to carbon monoxide and dioxide and oxidation of hydroxyl to oxygen. The heterogeneity of the metal and adsorption of formic acid on active centres[2,3] and the formation of complexes between titanium and formic acid may also contribute to the overall process.

The reason for the formation of barrier films at more elevated temperatures and at higher acid concentrations (Fig. 1, zone *A*) may involve a consideration of the kinetics of oxidation of formic acid and the properties of the anodic oxide film under the conditions specified.

The phenomenon of micropitting appears to be confined to the titanium–formic acid system as similar results could not be achieved with niobium or zirconium.

It is considered that further work on this phenomenon may contribute to an understanding of the general mechanism of the pitting corrosion of metals.

The anodizing of titanium in formic acid to produce a micropitted surface as an aid to anti-galling, lubrication and adhesion of surface coatings is covered by a patent application[4] made by the National Research Development Corporation.

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Some New Intermetallic Compounds of Thorium of the Type Th_7X_3

As part of an investigation of the constitution of alloys of thorium with the metals ruthenium, rhodium, palladium, osmium, iridium and platinum, a series of 1-gm. samples of alloys containing up to 33 atomic per cent of each precious metal was prepared by arc-melting the component materials in a zirconium-gettered argon atmosphere, weight losses on melting being negligible. Metallographic and X-ray results on as-cast and on annealed material suggest that the intermetallic compounds richest in thorium are: Th_7Ru_3, Th_7Rh_3, Th_2Pd, Th_7Os_3, Th_7Ir_3 and Th_7Pt_3. Each compound forms a eutectic with thorium and the composition and temperature of these eutectics are given in Table 1. The solid solubility of each metal in thorium and the age-hardening behaviour of thorium-rich alloys have also been investigated.

Alloys corresponding to the five compounds, Th_7X_3, were brittle and powders for X-ray studies were prepared by crushing in air; powder patterns were obtained with a Guinier-type focusing camera using monochromatized copper radiation. These X-ray films were indexed on the basis of a hexagonal lattice, systematic extinctions of the $h\,h\,\overline{2h}\,l$ reflexions were noted when l was odd and the observed intensities show good agreement with those for the Th_7Fe_3 structure, space group $C^4_{6v} - P\,6_3mc$. Lattice parameters, axial ratios and X-ray densities for the new compounds are given in Table 2.

Table 1

System	Phases in the eutectic	Composition of the eutectic (percentage of precious metal)	Melting point of the eutectic (deg. C.)
Th – Ru	Th + Th_7Ru_3	16 ± 1	1,262 ± 12
Th – Rh	Th + Th_7Rh_3	20 ± 1	1,237 ± 12
Th – Pd	Th + Th_2Pd	22 ± 1	1,112 ± 12
Th – Os	Th + Th_7Os_3	14 ± 1	1,287 ± 12
Th – Ir	Th + Th_7Ir_3	15 ± 1	> 1,300
Th – Pt	Th + Th_7Pt_3	17·5 ± 0·5	1,242 ± 12

Table 2

Compound	'*a*' (Å.)	'*c*' (Å.)	*c/a*	X-ray density (gm./c.c.)
Th_7Ru_3	9 969	6·302	0·632	11·82
Th_7Rh_3	10 031	6 287	0·627	11·71
Th_7Os_3	10 031	6 296	0·628	13·27
Th_7Ir_3	10 076	6 296	0·625	13·40
Th_7Pt_3	10 126	6 346	0·627	13 01

Grateful acknowledgment is made to Prof. J. G. Ball under whose supervision this work was carried out, and also to the Atomic Energy Research Establishment, Harwell, for financial support and for allowing me to make use of some experimental facilities.

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CRYSTALLOGRAPHY

X-Ray Correlation of the *A-B* Layer Order of Cadmium Selenide with the Sign of the Polar Axis

Cadmium selenide (CdSe) is typical of the hexagonal semiconducting compounds formed from elements of Group II and Group VI of the Periodic Table. It possesses the wurtzite structure and contains a unique polar axis (the *c*-axis). Compression along this axis produces opposite electric charges on the basal faces, which also differ in their response to etching and in other properties. An examination of the crystal structure as shown in Fig. 1 shows the polarity clearly. Viewed normal to the *c*-axis, pairs of closely spaced cadmium–selenium layers are seen. The 'cadmium side' and the 'selenium side' are defined in Fig. 1.

For an understanding of the polar physical and chemical effects it is important to know which is the cadmium and which is the selenium side of the *c*-axis. It is possible to make such an identification by observing the effects of anomalous X-ray dispersion on the intensity of the X-rays reflected from the positive and negative sides of the basal planes $(00.l)$ and $(00.\bar{l})$. Previous workers have investigated cubic zinc sulphide, gallium arsenide and indium arsenide in such a manner[1-3]. To our knowledge, this communication reports the first application of this

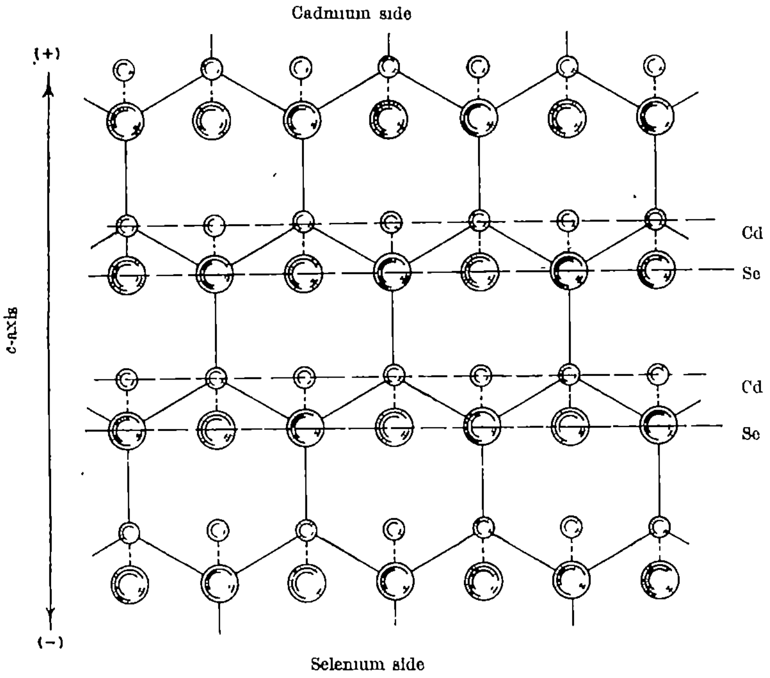

Fig. 1. Alternate layers of cadmium and selenium parallel to the (00.1) surfaces

technique to a wurtzite structure. By making dispersion corrections to the atomic scattering factor, the structure factor ratio $\frac{|F(00.l)|^2}{|F(00.\bar{l})|^2}$ (which is proportional to the ratio of the integrated intensities) can be calculated[4]. These theoretical ratios calculated for copper $K\alpha$ radiation are shown in Table 1.

Table 1. ROTATED INTEGRATED INTENSITY RATIOS

Plane	Theoretical intensity ratio $\frac{\lvert F(00.l)\rvert^2}{\lvert F(00.\bar{l})\rvert^2}$	Observed intensity ratio
00.2	0·860	0 862
00.4	1 000	0 973
00.6	1·225	1 241
00.8	1·000	1 045

The experimental procedure is quite exacting. A circular plate of cadmium selenide, the surface of which was orientated to within 3 min. of arc with the (00.1) plane, was etched and given an optical polish with 0·3μ alumina. Measurements with an optical flat indicate that both surfaces were slightly rounded but were flat to 10 fringes. Next, the disk was placed on a bed of wax in a cylinder which was inserted into the rotating flat specimen spinner of a standard 'Norelco' diffractometer. The polished crystal was pushed up against an optical flat which was held against the top surface of the specimen holder. When the interference rings were centred, the crystal was locked into position. To test the parallelism of the crystal surface with the plane of the specimen holder, the latter was rotated by motor while a distant light source was reflected off the rotating crystal surface. The steadiness of this optical image proved to be an extremely sensitive test of the line-up. The experimental values for the integrated intensities were obtained by counting while simultaneously rotating the crystal at 77 r.p.m. and slowly scanning the entire peak at 1/8° (2θ) per min. After the rotated integrated intensities were measured on one side, the crystal was turned over, aligned, and the same process was repeated (care being taken that all conditions were identical for any two $(00.l)$ $(00.\bar{l})$ reflexions). For different peaks various aluminium filters were used so that the counting-rate fell within the linear response range of the scintillation counter. Table 1 compares a typical set of results with the corresponding theoretical values.

The side of the crystal proved to be the cadmium side by the X-ray data of Table 1 was found to become positively charged on extension. By the sign convention of the I.R.E. 1949 Standard on Piezoelectricity the + end is therefore the outward directed normal from the cadmium side.

Having established this correlation, other differentiating properties of opposite (00.1) surfaces which have been correlated to the piezoelectric sign can also be related to the *A*–*B* layer order. Table 2 is a tabulation of some of these correlations for cadmium selenide.

Considering the similar character of all wurtzite and sphalerite compounds, it would appear reasonable for these compounds to show the same type of correlation as cadmium selenide, namely, the more metallic layer will be in the +c direction, as defined by the piezoelectric sign. This is in keeping with the findings for zinc sulphide and (by inference from the etching behaviour) for gallium arsenide and indium arsenide[1–3]. Any theory which attempts to explain the differentiating properties of the opposite (00.1) surfaces of wurtzite and the opposite (111) surfaces

Table 2. CORRELATION OF *A*–*B* LAYER ORDER WITH OTHER PROPERTIES WHICH DISTINGUISH THE OPPOSITE (00.1) SURFACES OF CADMIUM SELENIDE

Property	(00.1) surface. Outward normal to surface points in + *c* direction	(00.1) surface. Outward normal to surface points in − *c* direction
Sign of piezoelectricity on extension	(+) As defined by 1949 I.R.E. convention	(−)
A–*B* layer order	See Fig. 1	
Etching characteristics in aqua regia (excess selenium removed)	Shiny, symmetric pits bevelled edges Equal or slower etch rate	Dull or cratered, no symmetry pits, no edge bevelling. Equal or faster etch rate
Relative sign of electrolytic solution potential 1 : 1 aqua regia	(+) More 'noble' by 50–70 mV	(−)
Crystal habit at positive and negative ends	More complex pyramid development	Prominent face is the pedion. Pyramids small or absent

for sphalerite compounds must be compatible with these findings.

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OCEANOGRAPHY

Organic Acids in Pacific Ocean Waters

Organic acids dissolved in waters of the Pacific Ocean were isolated by : (1) solvent extractions with carbon disulphide and chloroform, and (2) by co-precipitation with ferric hydroxide sols.

The solvent extractions were carried out on 15·3–20·0 l. of sea-water samples freed of particulate matter by filtration through '*HA* Millipore' filters (pore size : 0·45μ). The residues from chloroform and carbon disulphide extracts were methylated with methanol and a trace of concentrated sulphuric acid, and these products were then submitted to gas chromatographic analysis using an 'Aerograph' model 110-*C* gas chromatograph (Wilkens Instrument Co., Walnut Creek, California) incorporating 5- and 10-ft. Craig polyester succinate columns. The fatty acid esters isolated were identified by their retention times on the column with respect to known fatty acid esters and in the case of the methyl esters of palmitic and stearic acids by collection from the column and subsequent infra-red spectra. Table 1 lists the fatty acids isolated in μgm./l., and includes pertinent data on sample location and depth, extraction *p*H, and indicates the amounts of the unknown fatty acids C_x, C_y and C_z. From their infra-red spectra, C_x, C_y and C_z are indicated to be fatty acid esters. Their relative positions on the gas chromatographic traces gave retention times identical with the C_{15}, C_{17} and C_{19} normal, saturated fatty acid esters. Fig. 1 illustrates two representative traces for samples 47-*A* and 10-*A*.

To determine the percentage recovery of the fatty acids from sea-water a tracer experiment was run using palmitic acid-1-^{14}C (Nuclear-Chicago, Cat. No. *CFA*-23), added as 2,345 c.p.m. to each 2·40–2·48 l. sample of filtered sea-water prior to extraction with chloroform. The samples were acidified with hydrochloric acid to a *p*H of 2·0, and the solvent

Table 1. Fatty Acids isolated from Sea-water

Sample (1)	Location	Depth collected (m.)	Depth to bottom (m.)	Volume extracted (l.)	Extraction (*p*H)	μgm./l. Saturated C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	Unsaturated Mono- C_{16} (C=C)	C_{18} (C=C)	di- C_{18} $(C=C)_2$	Unknown C_x	C_y	C_z	Miscellaneous	No. of major peaks (5)	Total (μgm./l.) (3)
10*A*	31° 40′ N. 119° 35′ W.	1,000	3,290	19 0	1 30	2 3	4 3	20·9	14·2	1 8	1 4	0·3	8·3	1 4	—	—	—	1·6 (4)	13	56 5
55*A**	32° 40′ N. 118° 09′ W.	1,200	2,020	18·1	3·10	0·4	1·8	14·6	22·2	8·1	tr.	—	—	—	6 6	15 5	14·1	6 4	14	89 7
47*A**	32° 40′ N. 118° 09′ W.	1,500	2,020	19 0	2·88	0·2	1·8	7 9	14·0	7 0	tr	—	—	—	3 3	10·1	9 2	7·4	14	60 4
127*B*	25° 19′ N. 114° 05′ W.	1,957	4,070	20 0	2 30	0 3	2·5	12 2	17·7	5·4	—	—	—	—	6·2	13·1	10·1	4·2	14	71 7
34*A*	46° 46′ S. 123° 57′ W.	2,427	4,330	15·4	2 28	1 5	1·6	8·2	7·1	tr.	—	—	—	—	tr.	tr.	tr.	6·7	10	20 1
37*A*	26° 45′ N. 123° 00′ W.	987	4,280	20 0	7 50	0 7	2 9	11 1	8 9	2 5	—	0 8	1·4	1·0	—	—	—	3 6	10	32 9
24*A*	31° 41′ N. 119° 35′ W.	1,020	3,570	19·0	7·50	0·5	1 9	8·3	6 5	1 5	—	3 0	7 5	2 9	—	—	—	0·1	10	32·2
35*A*	46° 46′ S. 123° 57′ W.	2,427	4,830	15·3	7·58	0 3	0·4	2 7	—	—	—	—	—	—	—	—	—	—	7	3 4
70*C* (2)	32° 40′ N. 118° 09′ W.	1,500	2,020	56	4 5	0·3	0·5	1 5	3·7	2·1	tr.	—	—	—	1·0	2 0	2 5	2 7	10	16·3

(1) Samples marked with asterisk were extracted with carbon disulphide; samples not marked, with chloroform.
(2) This sample had previously been precipitated with ferric hydroxide.
(3) These values represent minimum yields and are felt to be correct within a factor of two.
(4) Appears to be palmitolinoleic (hexadecadienoic) ester. Also shows indications of C_{16} and the unsaturated analogues of C_{12} and C_{14}.
(5) Identifiable peaks appearing on the gas chromatographic traces.

Table 2 Palmitic Acid-1-^{14}C Tracer Experiment

Sample (1)	Depth (m.)	Volume (l.)	Percentage of recovery free acid	Percentage of recovery methyl esters	Acids isolated as methyl esters (μgm./l. water) (3) C_{12}	C_{14}	C_{16}	C_{18}	C_{16} (C=C)	C_{18} (C=C)	C_{18} $(C=C)_2$	Total
Control (2)	—	2 40	75·7	13 0	3 3	4 4	6 6	—	—	—	—	14 3
1	10	2 48	97·0	8·9	1·9	3 3	24·8	—	—	—	—	30·0
2	91	2·44	96·4	12 5	4 1	5 0	28 5	7 5	18·2	11 1	10 2	84·6
3	460	2 44	96·4	10 9	2 7	3 9	17 4	14 5	8 7	11 4	11·7	70·3
4	924	2·40	98 1	14·0	1 8	6 8	41·0	16 5	3 5	34 8	17·3	111·7
5	1,333	2·45	90·0	19·1	2 0	5 6	18 2	25·6	—	—	—	51·4
6	1,853	2 47	91·6	9 8	—	—	30 4	—	—	—	—	30·4
7	2,491	2·40	80·5	5 2	—	—	35 8	—	—	—	—	35·8
8	2,665	2 42	81·2	6 1	—	13 9	19 1	—	—	—	—	33 0

(1) Location: 30° 23 4′ N.; 119° 13′ W. Depth to bottom · 3,660 m.
(2) 40 l. of double-distilled water previously found to contain traces of fatty acids.
(3) Corrected to 100 per cent yield on the basis of the percentage recovery of the methyl esters.

Table 3. Organic Acids isolated from Sea-Water by Co-precipitation with Ferric Hydroxide

Sample	Location	Depth collected (m.)	Depth to bottom (m.)	Volume extracted (l.)	Analysis (ash free)		Milligrams isolated		
					C	H	Total	Ash free	Per litre (ash free)
SFB—1	30° 49′ N. 119° 38′ W.	1,840	3,300	160	49 5	6·1	26 3	23·6	0·147
SFB—2	32° 45′ N. 121° 40′ W.	2,500	3,930	165	47·7	7·8	34·3	26·1	0 158
Control 1 (1)	—	—	—	—	—	—	2 0	—	< 0·011
Control 2 (2)	—	—	—	—	—	—	1·0	—	—

(1) Third re-precipitation of the original water from *SFB*—2 with ferric hydroxide and working up in the same manner as the original precipitates.

(2) Reagent control using distilled water and working up the ferric hydroxide precipitate as above.

extracts, freed of solvent, were plated on 1-in. copper planchets and counted with a Nuclear-Chicago model-*D*47 gas-flow counter. The free acids were then esterified and the methyl esters submitted to gas chromatographic analysis. The effluent methyl palmitate was collected from the column and counted to obtain the overall recovery. The yields and amounts of the saturated, mono-unsaturated and di-unsaturated acids based on the methyl palmitate recovery are shown in Table 2. There was no indication of the presence of the C_{15}, C_{17} and C_{19} esters in the tracer experiment.

The co-precipitation of organic acids with ferric hydroxide was effected by the initial addition of 10 mgm./l. of iron(III) as ferric chloride to each sample of filtered sea-water, previously acidified to a *p*H of 2–3 with hydrochloric acid. The ferric hydroxides sols were precipitated with 2 *N* potassium hydroxide to a final *p*H of 6·1–6·5, and the precipitate collected by centrifugation. This material was worked free of chloride ion, redissolved in hydrochloric acid to a *p*H of 2·0 and passed through 'Dowex-50' cation exchange resin (*H*-form, 200–400 mesh, 4 × cross-linked). The acids were eluted with 0·1 *N* hydrochloric acid, and the eluate adjusted to a *p*H of 3·5 with 2 *N* potassium hydroxide. The solution was taken to dryness *in vacuo* and the residual salts extracted repeatedly with *iso*propyl alcohol. The alcoholic extracts, dried and freed of potassium chloride, were worked up to give light yellow solids, showing blue-green fluorescence, and giving negative tests for halogens, phosphorus, nitrogen, boron, sulphur and iron. The yields and carbon–hydrogen analyses of this material from two stations are shown in Table 3. The ash-free material gave an average molecular weight of 395 and an equivalent weight of 208. Hydroxyl and carboxyl groups were indicated by the infra-red spectra (Fig. 2) and spot tests. Tests for unsaturation and aromaticity were negative. Ultra-violet absorption curves showed only increasing absorption from 700 to 220 mμ. Attempts to separate this mixture by paper chromatography and paper electrophoresis were unsuccessful.

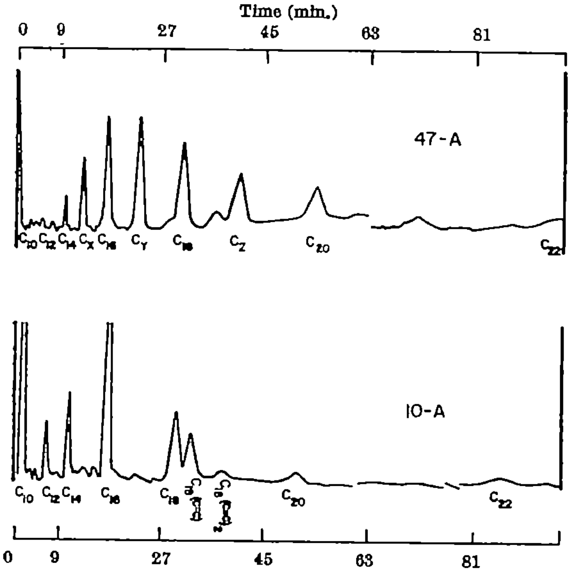

Fig. 1. Gas chromatographic traces of samples 47-*A* and 10-*A*

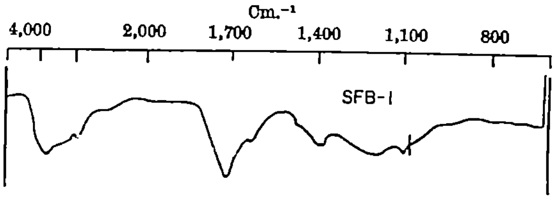

Fig. 2. Infra-red spectrum of *SFB*-1

A more detailed report and discussion of this work will appear elsewhere.

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BIOPHYSICS

Use of Potassium Tartrate for Equilibrium Density-gradient Centrifugation of Animal Viruses

Density-gradient centrifugation of both the velocity and equilibrium types has been applied for separating and purifying plant viruses such as tobacco mosaic and potato yellows[1], and also very recently to animal viruses such as Rous sarcoma[2], Shope fibroma[3] and polyoma[4]. Equilibrium banding with satisfactory recovery of biological activity has usually been achieved in rubidium or cæsium chlorides after centrifugation for approximately 24 hr. Some viruses are, however, inactivated by these salts, and others, as, for example, Rous sarcoma, require stabilization by albumin and citrate[2]. Non-equilibrium sedimentation in glycerol gradients has been used to purify Shope papilloma[3]; and reasonably sharp peaks of influenza and Newcastle disease viruses, and also of vaccinia hæmagglutinin, may be obtained after brief centrifugation in sucrose gradients of the velocity-sedimentation type[5].

In this communication we describe a simple method for equilibrium banding of some animal viruses, using preformed density gradients of neutral potassium tartrate. Equilibrium is obtained very rapidly in this salt, which possesses other advantages also in that concentrated solutions do not inactivate the infectivity or hæmagglutinins of the various viruses so far examined. Furthermore, the salt is inexpensive and no additional purification of the analytical reagent is necessary.

Gradients were prepared at 4° using two small mixing chambers, one of which was filled with concentrated potassium tartrate (varying from 35 to 60 per cent in different experiments), and the other with 2 per cent tartrate. The *p*H of the solutions was adjusted to 7·5. Virus samples (0·25–0·5 ml.) were then graded on to the tartrate gradient in an equal volume of the 2 per cent salt. Centrifugation was carried out in the Spinco *SW*39 head at 39,000 r.p.m. Most viruses banded satisfactorily in equilibrium after 1–2 hr.; but vaccinia hæmagglutinin, for example, may require 6 hr. centrifugation for close banding. After centrifugation, fractions were usually obtained by piercing the bottom of the centrifuge tube with a needle and running denser salt (70 per cent tartrate) up from below ('top unloader').

In some experiments the samples were collected by a novel method developed by one of us (R. S. E.), in which the centrifuge tube was lowered into a mixture of alcohol and dry ice and held there until the contents were frozen. The centrifuge tube was removed and cut rapidly into ten or more segments with a small saw. Each segment was then transferred to a tube and allowed to thaw. Contrary to expectation, control experiments showed that carefully controlled freezing did not distort or displace boundaries significantly, provided that the band was at least one-third of the way down the centrifugation tube. In several experiments, samples from equal volumes of virus centrifuged at the same time in the three buckets of the *SW*39 head were collected by three different methods, namely, dripping from the bottom of the tube, dripping from the top of the tube in the top-unloader, and by 'freezing-and-sawing'. In every instance the latter technique yielded as sharp, and frequently sharper, bands than those obtained by dripping. The freezing-and-sawing technique also offers the advantage that each fraction is collected independently, and there is thus less danger of contamination from one fraction to another than when all the samples are passed through the same orifice.

A typical sedimentation diagram from a sample of influenza *A* virus centrifuged in a tartrate gradient is shown in Fig. 1. It will be seen that after centrifugation for only 40 min. the majority of hæmagglutinin is recovered in equilibrium in a single fraction, and that only traces of activity are recovered in adjacent tubes of higher or lower densities. Equally sharp equilibrium peaks were obtained with influenza *B* and Newcastle disease viruses. In all cases the recovery of hæmagglutinin and infectivity was quantitative; and with Newcastle disease virus the titre of the most active hæmagglutinin fraction was actually from two- to four-fold higher than that of the original virus. The latter effect is presumably due to the removal of an inhibitor elsewhere on the gradient. The specificity of each hæmagglutinin was checked by neutralization with the appropriate antiserum. The most concentrated tartrate solution used (60 per cent) agglutinates a 1 per cent suspension of chicken erythrocytes to a dilution of 1 : 2–1 : 4 only.

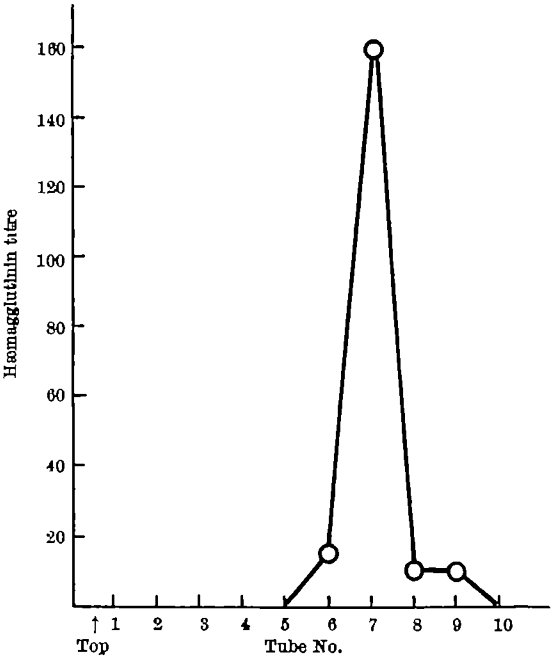

Fig. 1. Sedimentation diagram of influenza *A*, strain *MEL*, after centrifugation in a potassium tartrate gradient for 40 min. at 39,000 r.p.m. in the Spinco *SW*39 head. Maximum potassium tartrate concentration, 48 per cent. Virus diluted 1 : 2 in buffered normal saline, and 0·25 ml. placed on gradient

Potassium tartrate gradients are thus more convenient in many ways than gradients prepared with heavy salts such as cæsium and rubidium chlorides for equilibrium banding of several animal viruses. It seems likely that tartrate gradients could readily be applied for the concentration and purification of many other viruses and macromolecules, for the separation of virus mixtures, and for the separation of components from one virus. For example, as will be described in detail elsewhere, the vaccinia hæmagglutinin may be banded relatively sharply in 45 per cent potassium tartrate, whereas most of the infectious particles are found at the bottom of the gradient at this concentration[6]. Experiments are in progress in an attempt to sharpen the resolution of these and other viral components.

We are indebted to Dr. Carl Woese for valuable advice on gradient techniques. This work was supported by a research grant (*E* 2213-*C*2) of the U.S. Public Health Service, and by a grant from the Committee on Atypical Growth, Yale University.

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CHEMISTRY

Rapid Identification of High Polymers using a Simple Pyrolysis Unit with a Gas–Liquid Chromatograph

For some years it was standard practice in these laboratories to pyrolyse polymers in an intimate mix with ignited silver sand under carefully controlled conditions in an inert partial atmosphere. The degradation products were fractionated and examined by gas–liquid partition chromatography. The performance of the entire process in one stage was considered as a natural development of the technique, and the piece of apparatus to be described is now in regular use.

The considerations of design and operation of the pyrolyser were governed by the following factors: (*a*) The polymer degradation must be complete and should occur in the shortest possible time. A very thin film of polymer is therefore required and this will have the great advantage of minimizing secondary reactions between degradation products[1]. (*b*) 'Flash' depolymerization requires rapid heating of the very thin polymer film to the degradation temperature. This may be achieved by a large heating area relative to the mass of polymer. (*c*) Gaseous diffusion of the degradation products must be a minimum, therefore the pyrolysis coil must be in the chromatographic column itself and sited as near the surface of the packing material as is practicable. (*d*) To reduce the risk of condensation of any part of the gaseous sample the pyrolysis coil must be located within the column heater zone.

The pyrolyser (Fig. 1) consists of a rubber bung through which pass two pieces of 6 *BA* brass studding about 4·25 in. long; they project 1·5 in. above the bung and 2 in. below it. The upper ends are sleeved with polyvinyl chloride and terminate in a 5-amp. connector block, the lower ends are glass sleeved and terminate in screwed connector bushes. The pyrolysis coil itself is a 6-in. length of 28 standard wire gauge nichrome wire coiled to 4-mm. diameter over its middle 4 in., the two ends being fixed in the two screwed connector bushes. The associated electrical circuit is shown in Fig. 2.

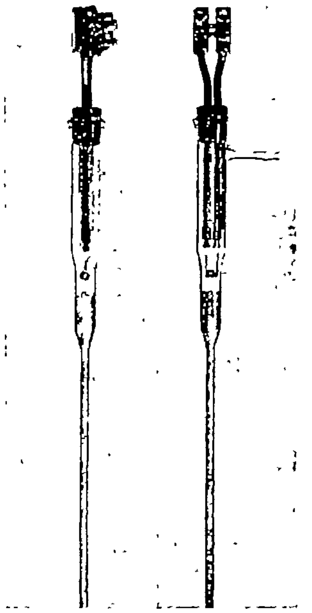

Fig. 1. Two views of the pyrolyser in position in a modified chromatographic column (quarter actual size)

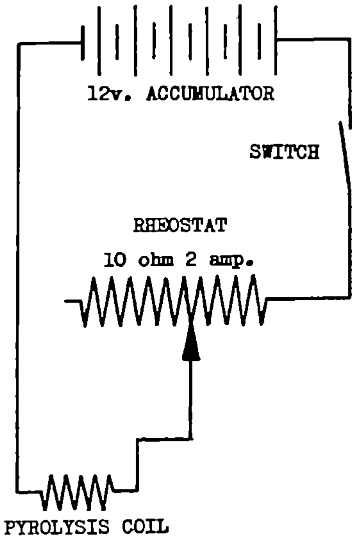

Fig. 2. Pyrolyser circuit diagram

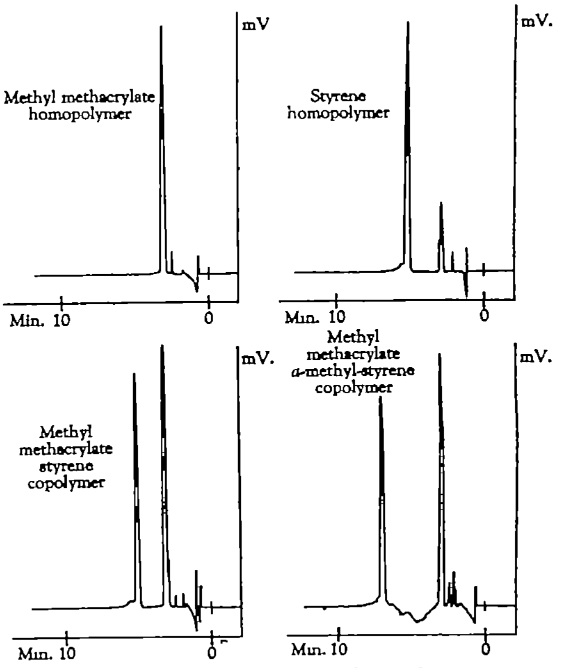

Fig. 3. Pyrolysis chromatograms of two homopolymers and two copolymers obtained on a 12·5 per cent polyethylene glycol succinate column operating at 80° C.

A very thin film of polymer, from a dilute emulsion or solution, or a dispersed gel, is cast on the filament, dried, and the assembly inserted in the top of a modified chromatographic column. The carrier gas flow is then started, and, after allowing a short time for the flow equilibrium to be set up, the coil is 'fired' at about 500° C. for 2–5 sec. The pyrolysis products are immediately swept on to the column by the carrier stream and chromatographed. Completeness of degradation can be checked by re-firing the coil at 1,000° C. when there should be no further signal from the detector due to degradation products.

Characteristic fingerprint chromatograms for homopolymers of methyl methacrylate and styrene, and copolymers of methyl methacrylate with styrene and methyl methacrylate with α-methyl-styrene, are shown in Fig. 3. When examining copolymers such as these, reliable quantitative assays of the monomers can be obtained, but with more complex monomers and co-monomers the method is only semi-quantitative.

Future modifications will include a precise method of filament temperature measurement, a filament of lower heat capacity—possibly in the form of a strip—and the development of a miniature version of the apparatus.

We wish to thank the Directors of Vinyl Products, Ltd., for permission to publish this communication.

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An Enrichment Trap for Use with Capillary Columns

In the course of an investigation into the volatile substances produced during the cooking of potatoes, difficulties were experienced when using gas chromatographic techniques[1] in the injection of small quantities of vapour. A nylon capillary column, length 50 ft., internal diameter 0·01 in., coated with 15 per cent tritolyl phosphate, and a Lovelock small detector[2] were used. It was found that the conventional injection by syringe into a 100 : 1 by-pass system did not allow sufficient vapour to pass into the column. The by-pass of argon carrier gas could be reduced to 30 : 1 without seriously impairing the resolution of the components being studied, but this still did not provide sufficient sample for detection. This method of injection was abandoned in favour of an enrichment trap. The enrichment trap technique for use with packed columns has been described[3].

The volatile substances given off from 1 kgm. of potatoes boiling in a 3-litre round-bottom flask were removed by a stream of nitrogen and freed from water vapour by passing successively through a glass U-shaped trap, cooled with solid carbon dioxide – acetone, and a similar trap cooled with liquid oxygen. The escaping volatile substances were then retained in a U-shaped trap consisting of 10 in. of 0·01 in. diameter nylon capillary coated with the same phase as the column. After collection for 20 min., the trap was disconnected and re-connected to the nylon capillary column (Fig. 1), while still immersed in liquid oxygen. With the carrier gas flowing, the trap was kept at – 183° C. until the air peak signal was obtained on the recorder, and then immersed in hot water at approximately 60° C. for 20 sec., removed, and left at room temperature. The chromatogram obtained showed a good separation of components, along with a small air peak which was a useful guide for obtaining relative retention times.

Confirmatory infra-red analysis cannot be obtained when using the nylon capillary column. The enrichment trap can, however, be used satisfactorily for mixed chromatography. The capillary trap is filled with unknown volatile substances as described above, then connected to the column as before and a small sample of authentic vapour, suspected to be present in the unknown, injected by syringe into the port situated in front of the 30 : 1 by-pass system. Thus 1/30th of the sample passes into the trap and is retained. The capillary trap is then disconnected from the column system and connected to a similar empty trap held in liquid oxygen. The original trap containing the unknown volatile substance and authentic sample is immersed in hot water causing the contents to mix intimately. A hyperdermic syringe needle is fitted to the first trap and an open syringe is used to propel the volatile substances from the first trap to the second trap. The second trap is then connected to the column as before and the contents chromatographed. The two-trap system is necessary since it was found that intimate mixing did not occur in the first capillary trap.

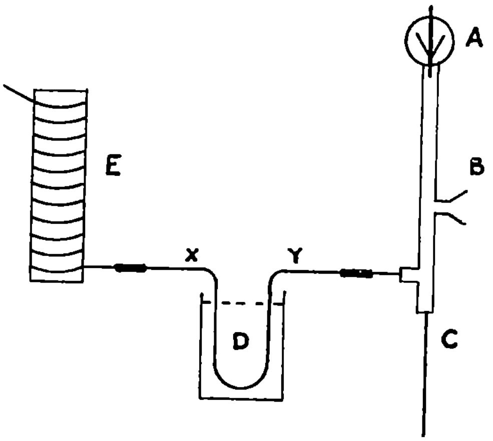

Fig. 1. *A*, Argon flow regulator; *B*, syringe injection port; *C*, nylon capillary by-pass; *D*, enrichment trap immersed in liquid oxygen; *E*, 50-ft. nylon capillary column. Volatile substances are collected in the trap with the gas flowing in an *XY* direction, but released as shown (ref. 3)

I wish to acknowledge the encouragement given by Mr. A. E. Joyce.

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Thermal Stability of Titanium Dioxide

Vorres and Dutton[1] and Vorres and Donohue[2] have recently described the preparation and crystal structure of titanium dioxide. Its decomposition in dry and moist air and dry nitrogen has been determined as part of our investigations of the thermal decomposition of some inorganic fluorides.

The results presented in Table 1 were obtained by the use of an atmosphere-controlled thermogravimetric balance the construction and operation of which have been described elsewhere[3]. The reported isothermal weight-loss data for runs 3 and 4, made in dry air and nitrogen, are maximum losses, and the X-ray examinations of their residues showed only titanium dioxide (anatase). Several additional experiments were carried out in a horizontal 'Vycor'

Table 1. Isothermal Weight-loss Data for Titanium Dioxide

Run No.	Temperature (deg. C.)	Atmosphere	Duration of the run (min.)	Total weight-loss (per cent)
1	340	dry air	4,285	47·0
2	400	dry air	1,350	54·9
3	460	dry air	112	60·0
4	440	dry nitrogen	393	59·9
5	340	moist air	330	22·3
6	400	moist air	186	24·2
7	455	moist air	88	28·5

tube at 460° C. with a stream of dry air flowing through the tube. In these experiments a white material condensed on a water-cooled cold finger placed 2 in. from the heated sample contained in a platinum boat. This condensate was very hygroscopic and reacted vigorously with water to form a clear, colourless, highly acidic solution which gave positive qualitative tests for both titanium and fluoride ions. These results agree well with the reported characteristics[4,5] of titanium fluoride. It was concluded that the fluoride formed during the decomposition of titanium dioxide and was swept away by the air stream (b.p. of titanium fluoride is 284° C.[5]).

From this information it was concluded that titanium dioxide in a dry atmosphere of air or nitrogen decomposed as follows:

$$2TiOF_2 = TiO_2 + TiF_4 \qquad (1)$$

The calculated loss of weight on the basis of this equation, if all the fluoride is lost, is 60·8 per cent, which is in good agreement with runs 3 and 4. Fig. 1 indicates that this decomposition begins at 150–200° C. and is complete by 500° C. when the heating-rate is 4° C. per min.

The weight-loss values for runs 5, 6 and 7 made in a moist atmosphere are maximum values. X-ray patterns of the residues show only titanium dioxide (anatase). From this evidence the simplest reaction is written as:

$$TiOF_2 + H_2O = TiO_2 + 2HF \qquad (2)$$

If only hydrogen fluoride is lost, the calculated loss of weight is 21·6 per cent. This value agrees well with run 5. However, the observed losses of runs 6 and 7 are noticeably greater.

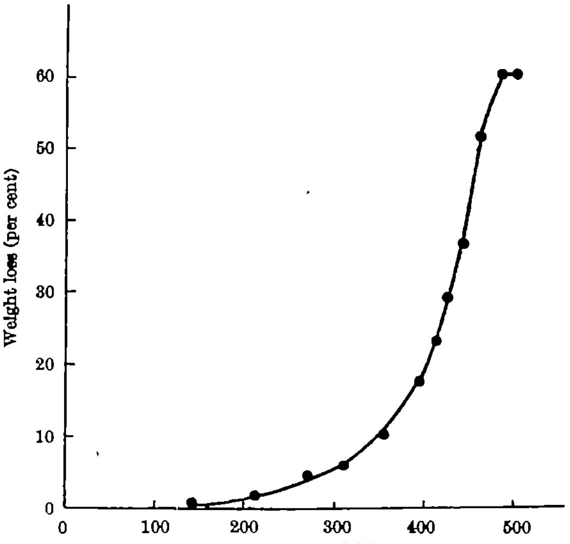

Fig. 1. Weight-loss of titanium dioxide heated at the rate of 4 deg. C. per min. in dry air

The following explanation for the observed deviations of runs 6 and 7 is suggested. Reaction (2) can be considered as the summation of the two reactions:

$$2TiOF_2 = TiO_2 + TiF_4 \qquad (3)$$

$$TiF_4 + 2H_2O = TiO_2 + 4HF \qquad (4)$$

If reaction (3) is the first step, then the loss of any titanium fluoride from the crucible before it is hydrolysed to titanium dioxide will cause a loss of weight in excess of that calculated for reaction (2).

It will be noted that the deviation from the expected loss on the basis of the simple one-step case (reaction (2)) increases for higher-temperature runs. At higher temperatures it was observed that the velocity of the decomposition was greater, so the probability of escape of titanium fluoride before it can react with water vapour in the atmosphere is also greater. This effect also depends, among other things, on the partial pressure of the water in the atmosphere, which was not considered.

A similar deviation with respect to increasing temperature was observed in the decomposition of Na_2SiF_6 in moist air[3].

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Ethyl Branching in the Fischer–Tropsch Synthesis

Current schemes for explaining chain-growth in the Fischer–Tropsch synthesis[1–4] do not permit the formation of an ethyl-branched hydrocarbon. We wish to report the identification of 3-ethylpentane in a hydrogenated Fischer–Tropsch synthesis product. A compound with this carbon skeleton has not hitherto been found in the product. Quantitative analyses by gas chromatography give a mole ratio of 2,3-dimethylpentane to 3-ethylpentane of 1·5. Weitkamp[1] found that 2,3-dimethylpentane constituted 0·9 volume per cent of the hydrogenated C_7-hydrocarbons from the synthesis product.

The Fischer–Tropsch product was made by passing 1 hydrogen : 1 carbon monoxide gas over a reduced-iron catalyst at 260° C., 21·4 atmospheres, and an hourly space-velocity of 300. The product was distilled to give four fractions, boiling from 60° to 170° C. These fractions were hydrogenated over platinum oxide catalyst. (It has been shown[4] that the distribution of skeletal isomers in the Fischer–Tropsch synthesis is identical for paraffins and olefins.) These hydrogenated fractions were chromatographed on Davison No. 950 silica gel, at column temperatures from − 10° to + 5° C., to isolate the hydrocarbon portions. Olefin-containing portions were re-hydrogenated and re-chromatographed. The combined saturated-hydrocarbon fractions were then distilled into narrow-boiling fractions.

Gas-chromatographic examination of these hydrocarbon fractions was made on a 26·5 ft. by 4·5 mm. internal diameter stainless steel column, coiled as a helix, containing 79 gm. of packing made in the weight ratio 12 parts tri-cresylphosphate to 88 parts 35–45 mesh 'Chromosorb'. By measuring the retention time relative to 2-methylhexane, a compound known to be present, 3-ethylpentane, was identified in these fractions.

Confirmation of 3-ethylpentane in these fractions was obtained by trapping-out the gas-chromatography peak at liquid nitrogen temperatures and analysing the 'freeze-outs' on a mass spectrometer. The 3-ethylpentane peak and part of the immediately preceding 3-methylhexane peak were in the freeze-outs. The hydrocarbon portions of duplicate freeze-outs analysed as 87 per cent 3-ethylpentane, 13 per cent 3-methylhexane, and 88 per cent 3-ethyl pentane, 12 per cent 3-methylhexane.

In the Fischer–Tropsch synthesis, 3-ethylpentane might be formed in any one of several ways: (1) The carbon chain might grow by adding a one-carbon unit to a methyl branch already present such as in the 3-methylpentane chain. (2) A one-carbon unit is added to 2-ethyl-1-butene (Weitkamp[1] shows that this is present in the product) by an oxo-type reaction. (3) Reaction occurs between a C_5-chain and a two-carbon unit, that is, 'multiple build-in' as suggested by Hall *et al.*[5]. (4) Isomerization of the carbon skeleton occurs to form 3-ethylpentane, but this seems unlikely.

This work is part of a larger project on the analysis of Fischer–Tropsch synthesis products through the C_7-compounds. The work in its entirety will be reported later. We wish to acknowledge the following who have contributed to this particular phase of the work: C. Zahn, G. Pantazoplos and A. G. Sharkey, jun.

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BIOCHEMISTRY

Hæmoglobins, Myoglobins and Erythrocruorins: a Proposal for modifying the Present Terminology

THE present terminology for those protohæm respiratory pigments which combine reversibly with oxygen, in which the terms hæmoglobin, myoglobin and erythrocruorin are used in varying senses by different authors, is far from satisfactory. Keilin and Hartree[1] showed that a convention introduced earlier by Svedberg[2], whereby all invertebrate pigments of this type were to be designated as erythrocruorins, was no longer justified on the basis of current knowledge. They suggested instead that 'hæmoglobin' should be used as a general term for both vertebrate and invertebrate pigments, and that the term erythrocruorin should be abandoned. According to their suggestion any given pigment should be identified by its origin, as in *Planorbis* hæmoglobin, muscle hæmoglobin, etc. It is with regard to the latter designation that this terminology immediately ran into difficulties.

I may recall that at the time when Keilin and Hartree made their proposal it was generally held that myoglobin (muscle hæmoglobin) and hæmoglobin in vertebrates were fundamentally different, and differences between them were always emphasized. Thus, Roughton, quite recently, commented[3] that: "It is an old creed that these two compounds are physicochemically similar, but this creed must now go, for the more intimately one studies them, the more frequent are the differences that one finds". Hence, Keilin and Hartree's proposal to equate myoglobins with hæmoglobins did not meet with general acceptance. However, following the work of Kendrew *et al.*[4,5], and of Perutz *et al.*[6], it might appear that such a proposal could now be defended. Yet the net result of the X-ray work, which proves that the tertiary structure of mammalian myoglobin and hæmoglobin is very closely similar, has been to demonstrate that there are at present no physicochemical criteria by which one can distinguish between these compounds in all cases. It might seem, therefore, that we should abandon the term myoglobin, and use instead the older names, muscle hæmoglobin or tissue hæmoglobin. Yet this is not so since there is a very real distinction to be made between the two, which is implicit in the term "tissue hæmoglobin". Hæmoglobins function in the transport of oxygen to the tissues and therefore occur in conjunction with some kind of circulatory system. They may or may not be found in corpuscles. Myoglobins are usually saturated with oxygen, remain within the tissues where they are located, and function to provide a reserve of oxygen against short-term deficits.

Keilin and Hartree summed up their intentions thus: "To avoid confusion and unnecessary complications of the nomenclature, the classification of all biologically active protein compounds, such as oxygen carriers or enzymes, should be based not upon their physico-chemical properties as proteins but upon their specific biological activities and the nature of their active, or prosthetic, groups". The distinction between myoglobins and hæmoglobins, made above, is in line with this aim. However, it is obviously unsatisfactory to use 'hæmoglobin' as a generic name for both groups of pigment. Furthermore, it would have the very real disadvantage that for many investigators 'hæmoglobin' means a compound with four hæm groups per molecule, with molecular weight 68,000, and all the other properties of mammalian hæmoglobins. Finally, one requires a term which carries no connotations of any sort other than that the pigment contains protohæm and combines reversibly with oxygen, for use in those cases where the given pigment cannot be classed as either myoglobin or hæmoglobin through lack of data; and possibly also if it should be found impossible to do so. For example, the recent work of Scholander[7] has raised the possibility, which seems especially attractive when considering certain invertebrate animals, that tissue pigments may act to promote the passage of oxygen to the tissues, rather than, or in addition to, carrying a reserve of oxygen. (The storage hypothesis has not found favour with investigators in certain specific cases, compare Eliassen[8].)

It is now possible to conceive a classification which takes account of these considerations, which has historical and logical justification, and which causes the least change to the existing terminology. The term 'erythrocruorin' (red pigment) was introduced by Lankester[9] as a general term for all protohæm oxygen-combining respiratory pigments, without any implication as to the structure or function of the pigment, and in contrast to the analogous pigments of certain marine worms which he called 'chlorocruorins' (green pigment)—a term which is still used in this sense[10]. It is therefore proposed that the term erythrocruorin be used for all known or presumed protohæm respiratory pigments which are known or presumed to be capable of undergoing reversible oxygenation. All erythrocruorins which are known or presumed to function in oxygen storage can be classed as myoglobins. It will be noted that this scheme maintains the present usage for vertebrate erythrocruorins, but also incorporates it into a general scheme for both vertebrate and invertebrate pigments.

It may be noted also that the prefix 'myo-' could possibly be used for pigments other than erythrocruorins which are analogous to myoglobins. Manwell[11] has already used the term myohæmerythrin in this sense.

Consistent use of the terminology suggested here will be found to lead not only to greater flexibility in discussion but greater precision also. It is hoped that these advantages, together with those enumerated above, will commend themselves to other workers in the field.

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Rehardening of Softened Enamel Surfaces of Human Teeth by Solutions of Calcium Phosphates

Rehardening of softened human enamel has been attempted in the past[1-3], but has not been clearly demonstrated since the earlier workers were not able to show that the softened material was not lost. Knoop hardness measurements provide a quantitative and sensitive measure of surface changes, and any loss of surface can be shown by alterations of the size and shape of the previously placed indentations. This communication describes conditions which have been developed for rehardening tooth surfaces, softened by a prior treatment with a buffer.

A Kentron microhardness tester was used with Knoop diamond indenter, as described previously[4,5], for following changes in surface hardness. Human teeth were tested for hardness initially, after softening by acetate buffer, and after treatment in calcium phosphate solutions. For each determination, ten indentations were made, and the hardness was expressed as an average Knoop hardness number. The initial indentations were often remeasured at the end of the experiments, and no change of length was noted. Softening of the teeth was produced in groups of ten by treatment with 1 litre of acetate buffer 0·001 *M*, *p*H 5·50, for 2–12 hr. at 37°.

For the preparation of rehardening solutions, two calcium salts were used, secondary calcium phosphate dihydrate (calcium/phosphorus molar ratio, 1·0) and synthetic hydroxyapatite (Ca/P ratio, 1·67). A weighed amount of salt (50 m.moles of calcium) was dissolved in twice this molar concentration of hydrochloric acid, and the volume was made up to 1 litre. From this stock solution, aliquots corresponding to 0·75 or 1·25 m.moles of calcium were pipetted into bottles containing distilled water at 37°. Aliquots of potassium fluoride stock solution (0·02 *M*) corresponding to 1–7 p.p.m. were added, if desired. The volume was made up to about 480 ml. with distilled water and sufficient potassium hydroxide (0·05 *M*) was added to bring the *p*H to predetermined values. The final volume was adjusted to 500 ml. The solutions were metastable, the *p*H dropped during the period of treatment, and some precipitation occurred.

Softened teeth were kept in pairs in 500 ml. of the rehardening solutions at 37° C. without stirring, and their hardness was determined at various time-

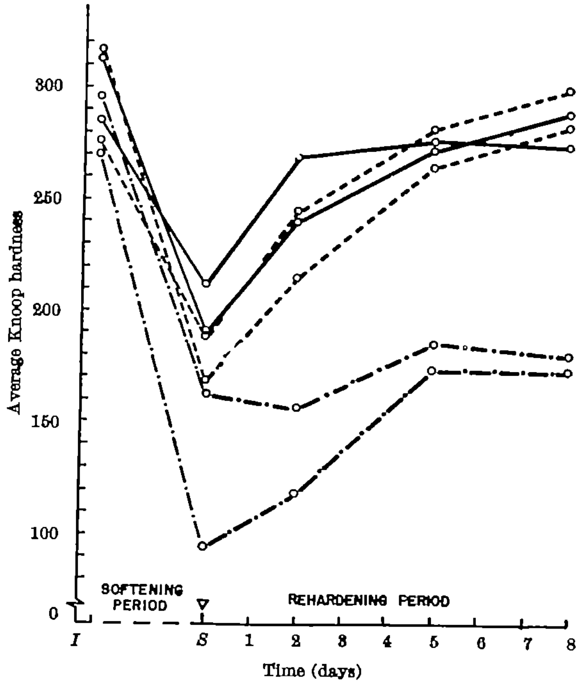

Fig. 1. Rate of rehardening of softened enamel surfaces as related to the extent of softening. *I*, Initial hardness; *S*, hardness after treatment with acetate buffer for 2 hr.: ——; 4 hr., – – –; 8 hr., — — —. Rehardening solution: Ca, 2·5 m.moles/l.; Ca/P, 1·0; *p*H 7·0; changed every 24 hr.

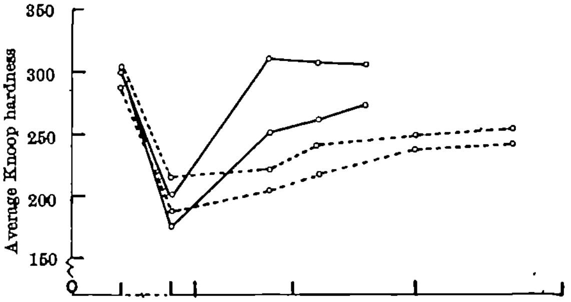

Fig. 2. Effect of fluoride on the rate of rehardening of softened enamel surfaces. *I*, Initial hardness; *S*, hardness after softening in acetate buffer for 4 hr. 1 p.p.m. fluoride, ——; no fluoride, – – –. Basic rehardening solution: Ca, 1·5 m.moles/l.; Ca/P, 1·67; *p*H 7·3; changed every 2 hr.

intervals as indicated in Figs. 1 and 2. The results in this communication are representative of rehardening experiments involving a total of more than 300 teeth.

Fig. 1 gives the results for individual teeth obtained with solutions prepared from the secondary calcium salt (Ca/P ratio, 1·0) and changed daily. Preliminary results of this type were reported earlier[6]. Four of six teeth showed a return to the initial hardness after treatment for 8 days. The two teeth that did not show considerable rehardening were those softened for 8 hr. to a low hardness. This and other similar results showed that complete rehardening did not occur if the softening process had proceeded too far, below about 150 Knoop hardness number. This limit may be of considerable interest in that it may be the boundary between sound and carious enamel and represent the limit of possible natural reparative recalcification of enamel. Sobel and his associates[7] have shown that calcification takes place in two discrete stages, nuclei formation and subsequent crystal growth. The remineralization of these experiments may refer only to the stage of crystal growth.

The rate of rehardening was found to be more rapid for solutions with Ca/P ratios of 1·67, especially in the presence of small amounts of fluoride. The results are shown in Fig. 2. The teeth kept in 500 ml. of solution containing 1 p.p.m. of fluoride regained the initial hardness in 4 hr., whereas in the absence of fluoride the recovery was much slower.

The accelerating effect of fluoride ions on the rehardening of enamel adds a second explanation for the mechanism of fluoride protection from dental caries. In addition to producing more insoluble enamel, fluoride ions appear to affect the dynamic equilibrium between tooth surfaces and the oral fluid by accelerating the enamel remineralization.

Table 1. INHIBITION OF *in vitro* CARIES IN THE ARTIFICIAL MOUTH IN THE PRESENCE OF REHARDENING SOLUTIONS
(Calcium/phosphate, 1·67; calcium, 1 5 m.moles/l. fluoride, 0·3 m.moles/l., *p*H 7 1. Freshly prepared medium was used twice daily)

Hardness (Knoop hardness number)* Experimental (Culture medium + rehardening solution)		Control (Culture medium alone)	
Initial	After 5 days	Initial	After 5 days
188	176	235	86
226	205	282	81
262	269	261	96
278	262	249	82
295	283	273	29
298	285	291	20

* Average *S.D.* ± 14

The rehardened enamel was tested for resistance to abrasion by tooth brushing and for resistance to softening by a second exposure to the acetate buffer (*p*H 5·50) for 2–4 hr. Brushing caused no appreciable loss of surface material. For 34 teeth, the average rate of softening by the buffer was 19 ± 3 Knoop hardness number/hr. for the rehardened teeth as compared with 26 ± 6 Knoop hardness number/hr. for the original surfaces.

In the artificial mouth[8], teeth exposed to oral bacteria and a culture medium supersaturated with calcium phosphate and containing 6 p.p.m. fluoride were essentially unaltered after 5 days exposure (Table 1). The controls were softened to the limit of the sensitivity of the measurements.

This evidence for enamel rehardening emphasizes the dynamic nature of the enamel surface and the importance of the oral fluid in the carious process. Prevention and healing of initial carious lesions seem possible by modification of the composition of the oral fluid either systemically or locally.

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Biliverdin in the Eyes of *Polyphemus pediculus* (L.) (Crustacea, Cladocera)

THE large median compound eye of the Cladocera begins its development as two separate rudiments which enlarge gradually and fuse in the mid-line. In the majority of Cladocera these rudiments are red in colour and gradually darken so that they are black just before fusion. In embryos of *Polyphemus pediculus* the eye rudiments are green in colour, and when treated with yellow nitric acid they give a positive Gmelin reaction, indicating the presence of a bile pigment.

A large sample of female *Polyphemus*, containing numerous embryos, was first extracted with acetone to remove carotenoids and then extracted with acid methanol (5 per cent hydrochloric acid), giving a deep olive-green solution. When this was diluted with water and shaken with chloroform the chloroform phase became blue in colour while the acid methanol phase became orange-brown. The chloroform phase was washed with dilute sodium bicarbonate, then with water and evaporated to dryness. When the pigment was redissolved in acid methanol the absorption spectrum showed a sharp peak at 374 mμ and a lower, more rounded, peak at 685 mμ. These are close to the absorption maxima of biliverdin hydrochloride; the small differences can be attributed to the presence of other substances in the extract.

The identity of the green pigment as biliverdin was confirmed by the addition of zinc acetate and a little iodine to a methanol solution in the presence of a trace of ammonia. The result was a green solution with a brilliant pink fluorescence in ultra-violet light, and a sharp absorption band at 635 mμ showing that the pigment had proto not meso side-chains.

The presence of biliverdin in the eyes of adults was demonstrated by selecting females without embryos and extracting with methanol. The addition of zinc acetate and iodine produced the same results as with the larger sample.

It has not been possible to demonstrate a Gmelin reaction in the eyes of adult *Polyphemus* because of the presence of large amounts of a dark masking pigment. This pigment is also extracted with acid methanol, but remains in the methanol phase when it is diluted and shaken with chloroform. Further studies are being made of this and similar pigments in other Cladocera; meanwhile, it is possible to say that they are similar in many respects to ommochromes, being insoluble in most organic solvents, but easily soluble in acids, and showing reversible changes in colour according to whether they are oxidized or reduced.

Biliverdin is rare in the Crustacea; it has been found only in the digestive gland of the crayfish *Cambarus*[1], and in the roots of certain parasitic cirripedes[2]. The present record of biliverdin in *Polyphemus* adds another crustacean order to the few known to include bile pigments in their metabolism[3]. It is interesting in this connexion to note that, although searched for, no bile pigments have been found in the cladoceran *Daphnia*[4].

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PHYSIOLOGY

Magnesium Absorption in Sheep

Low levels of dietary magnesium precipitate hypomagnesæmia experimentally in lambs[1]. It is not known whether these findings are related to the hypomagnesæmia which occurs spontaneously in adult ruminants near parturition in autumn and spring on some pastures. The present communication reports an attempt to produce hypomagnesæmia in sheep, but with a different type of roughage-containing diet.

Lambs born the previous year were fed the experimental rations beginning in June. Sheep 1 (Border × Clun cross) had previously been on a high plane of nutrition, that is, good pasture; sheep 2 (Border × Suffolk cross) had been on a low plane of nutrition, that is, stubble. A diet relatively low in magnesium was fed (Table 1) and distilled water supplied for consumption *ad libitum*. The calcium intake was kept at a high level, since an inter-relationship between calcium and magnesium availability is well substantiated. The magnesium content of the diet was higher than had been hoped probably due to the inclusion of mineral salts from tap-water used to wash the fodder cellulose prior to the drying process.

Table 1
Chemical analysis (dry-weight basis)

Feeding stuff	Magnesium (per cent)	Calcium (per cent)	Phosphorus (per cent)
Fodder cellulose	0·16	0·825	0·063
Molasses	0·28	0·94	trace
Flaked maize	0·109	—	0·195
Lucerne hay	0 233	1 97	0·288
$Ca_3(PO_4)_2$	—	38·7	20·2
NaCl	—	—	—

Mineral composition of daily ration during test period
(dry weight basis)
Sheep 1. (Final live weight = 96 lb.)

Feeding stuff	Amount fed (gm.)	Total magnesium (gm.)	Total calcium (gm.)	Total phosphorus (gm.)
Fodder cellulose	460	0·736	3·80	0·29
Molasses	23	0·065	2·15	—
Flaked maize	174	0 190	—	0·84
Lucerne hay	428	0·997	8 44	1·23
$Ca_3(PO_4)_2$	9	—	3·48	1·82
NaCl	9	—	—	—
Total per day		1·988	17 87	3·68

Sheep 2. (Final live weight = 66 lb.)

Feeding stuff	Amount fed (gm.)	Total magnesium (gm.)	Total calcium (gm.)	Total phosphorus (gm.)
Fodder cellulose	276	0·442	2·28	0·174
Molasses	23	0·065	2·19	—
Flaked maize	87	0·095	—	0·170
Lucerne hay	342	0·798	6 75	0·987
$Ca_3(PO_4)_2$	9	—	3·48	1·820
NaCl	9	—	—	—
Total per day		1·400	14 70	3 151
During urea feeding (+ 184 gm. cellulose)		(1·72)	(16·22)	(3·097)

The overall percentage of magnesium in the ration was about 0·18 per cent (dry weight basis) compared with 0·02 per cent (air-dry basis) used by other workers[1] and hypomagnesæmia was not observed in the sheep (Table 2). Feeding stuffs were analysed, after ashing, for calcium[2] and magnesium[3]; total phosphorus determinations on feeding stuffs followed wet oxidation with sulphuric acid/hydrogen peroxide[3]. The same methods were applied directly to blood serum.

Table 2. BLOOD SERUM MAGNESIUM, CALCIUM AND INORGANIC PHOSPHORUS (MGM./100 ML. SERUM)

	Magnesium				Calcium				Inorganic phosphorus			
	A	*B*	*C*	*D*	*A*	*B*	*C*	*D*	*A*	*B*	*C*	*D*
Sheep 1	1·9	2·5	2 1	2·2	11·6	11·0	10·8	10·7	5·6	5·3	4·9	6·1
Sheep 2	2·0	2·3	1·9	2·0	9·9	9·2	9·5	8·9	7·4	7·1	6·2	8 2

A, Basal sample; *B*, low-magnesium ration for 19 days; *C*, low-magnesium ration for 36 days; *D*. low-starch low-magnesium ration with urea for a further 29 days.

A comparison of the diets used in different experiments is of interest. Recently it has been suggested that dietary phytate may inhibit magnesium absorption from the intestine of the rat[4]. The use of corn cobs by McAleese and Forbes in the diet of the lambs and in the bedding[1] would imply a high intake of phytate compared with the small amounts of phytate provided by flaked maize and lucerne hay in the diet listed in Table 1. The competition of phytate for magnesium may exacerbate the already low-magnesium intake, as in the experiments of McAleese and Forbes[1], and so account for the very rapid onset of hypomagnesæmia. Hence the possibility that dietary phytate is a factor in spontaneous hypomagnesæmia needs to be examined, as this competitive effect may occur in the ruminant as well as in the monogastric animal.

A further known factor concerned with magnesium absorption in cows is the level of rumen ammonia[5]. Introduction of large amounts of ammonium acetate and carbonate into the rumen via a fistula reduced both the urinary magnesium excretion and the serum magnesium-levels. Feeding 10 gm. of urea daily, which presumably forms ammonia *in vivo*, did not lead to hypomagnesæmia in sheep on a reduced magnesium intake (Table 2). The rise in serum inorganic phosphorus-level after urea feeding is unexplained; release of phosphate previously bound to phytate would certainly increase the effective phosphorus intake. Flaked maize was omitted from the diet during urea feeding since low-starch rations favour urea toxicity and presumably ammonia production[6].

Only an approximate comparison of the relative amounts of ammonia supplied can be made. Ammonium acetate or carbonate was administered to cows via rumen fistulæ at a rate of about 20–30 gm. ammonia per 100 lb. body-weight[6] compared with about 6–9 gm. ammonia per 100 lb. body-weight given as urea to the sheep.

This evidence reinforces current theories that spontaneous hypomagnesæmia is more complex than a simple nutritional deficiency. Environmental, genetic and endocrine factors may also be concerned in the aetiology of this aberration.

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Bioassay of Ciguatera Toxin

The tropical fish poisoning known as 'ciguatera', which is clearly different from the well-documented tetraodon poisoning, has long been known in the Pacific. Its narrow regional appearance and its variability within a given species of marine fish have been puzzling phenomena. The symptoms associated with ciguatera have been well described, but fundamental knowledge of the toxin is virtually non-existent. The present state of this problem has recently been reviewed by Randall[1].

As the first stage of an investigation aimed at the isolation and identification of the toxic principle, it was necessary to develop a reliable bioassay. The bioassay which had been devised for *Gonyaulax* toxin from shellfish[2] was used and standardized for ciguatera toxin[2] by Halstead and Bunker[3]. A re-investigation of this method in this Laboratory proved that this assay could not be employed with confidence and emphasized the necessity of finding an unambiguous bioassay. As is shown by the results in Table 1 aqueous extraction of toxic fish as described by Halstead and Bunker[3] failed to extract the toxin. It was found that fish or fish extract could be screened for toxicity by feeding raw fish or extract residue mixed with egg to the mongoose (*Herpestes mungo*). This animal exhibits symptoms not unlike those which have been described for humans following ciguatera poisoning. With this qualitative assay it could be shown that ethanol will extract efficiently the toxin from *Lutjanus bohar* (Forskål) collected in the Line Islands and known to be toxic as a result of mongoose feeding. The extraction residue after removal of the ethanol *in vacuo* was injected intraperitoneally into mice. While such an extract would kill mice, so would a similar extract of a non-toxic control fish *Pristipomoides microlepis* (Bleeker), although at a higher dose-level. Satisfactory differentiation of toxic and non-toxic fish could be achieved with a more highly purified extract. The initial ethanolic extract of dried fish flesh was concentrated *in vacuo* and diluted with thrice its own volume of water. The resulting solution was first extracted with light petroleum, then with ether. The bulk of the toxin was found in the ethereal extract. This extract after removal of the solvent when injected intraperitoneally into mice (closed colony Carworth Farms Webster strain, weighing between 19 and 21 gm.) would produce kills at a level of 0·2 mgm./gm. while the ethereal extract of the control fish caused no symptoms at a level of 1·5 mgm./gm. The results of these experiments are included in Table 1.

While sufficient amounts of toxic extract for injection of larger numbers of animals have not been available for a quantitative bioassay, the assay method described above allows reliable recognition of ciguatera toxin. Further research aimed at the isolation and identification of the pure toxin is in progress.

Table 1. Comparison of Bioassay Methods

	Water extract (5 mice used for each specimen)	Intraperitoneal injection in mice: Diethyl ether extract, in mgm. extract/gm. mouse. Figures represent mean time to death in min. of two mice tested at each level					
		0·1	0·3	0·5	0·7	1·0	1·5
Specimen (*Lutjanus bohar*)							
Highly toxic							
1	*N.D.**	(348)†	189	130	63	—‡	—
2	*N.D.*	(1,627)	249	155	111	69	89
3	*N.D.*	*N.D.*	240	81	90	43	30
4	*N.D.*	(360)	90	44	63	62	43
5	*N.D.*	*N.D.*	261	139	78	72	49
6	*N.D.*	308	128	101	89	—	—
Moderately toxic							
7	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D*	*N.D.*	—
8	*N.D.*	*N.D.*	788	850	108	—	—
9	*N.D.*	*N.D.*	(2,800)	750	—	124	—
10	*N.D.*	*N.D.*	—	*N.D.*	—	(367)	—
11	*N.D*	*N.D.*	*N.D.*	1,252	—	1,044	—
12	*N D.*	*N.D.*	*N.D.*	(1,620)	—	—	—
Non-toxic							
13	*N.D.*	*N.D.*	*N.D*	*N.D.*	—	2,800	—
14	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—	*N.D.*	—
15	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*
16	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	1,000
17	*N.D.*	—	*N.D.*	*N.D.*	*N.D.*	*N.D.*	—
Pristipomoides microlepis (Control)	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D.*	*N.D*

* *N.D.* signifies no deaths in the 72-hr. test period.

† Figures in parenthesis are times to death in one mouse of pair tested, the other living through test period.

‡ Dash indicates no tests made at this level.

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Properties of Vessel Muscle Proteins extracted with Water or Salt Solutions of Low Ionic Strength

EARLIER investigations on the relations of the electrolyte content of organs to hypertension in the rat have shown that only the increase of the potassium content of the vessel wall is characteristic of hypertension[1]. In the carotic strip of cow *in vitro*, potassium produces a rise in tension which lasts so long as the potassium concentration of the bath solution is not altered. Simultaneously with the penetration of potassium into the muscle fibre a rise in tension[2], viscosity and dynamic elasticity modulus[3] occurs. It may be concluded from this that the potassium which entered the muscle fibre discharges certain proteins and so increases the mechanical tension.

Starting from this hypothesis, I have made extracts of relaxed vessel muscles using water or salt solutions of ionic strength either lower than that in the intracellular fluid or equal to it. The muscle of the relaxed cow carotis was separated from other tissues. Then it was minced in a small meat grinder, mixed in a mortar for 10 min. at room temperature with 1·5 c.c./gm. of the chosen solution and finally centrifuged for 15 min. at $22{,}000g$. Extracts so obtained have a high viscosity (η rel. $= 6\cdot02$ at a protein concentration of 1·55 per cent; extract of striated muscle of the same animal η rel. $= 1\cdot61$ at a protein concentration of 1·67 per cent) and show double refraction of flow. After addition of 0·6 *M* potassium chloride (*p*H 7·0) at 15–18° C., they turn into a more or less thixotropic gel after a few seconds. The gelling also takes place, but more slowly, at lower potassium concentrations so long as these exceed that in the intracellular space. Extracts prepared by the same procedure from striated muscle of the cow, rabbit or rat and from smooth muscle of the non-pregnant uterus, bladder and small intestine of the rabbit or the small intestine of the rat do not show the above-mentioned properties. (Extracts of smooth muscle of the bladder and small intestine of the cow show double refraction of flow.) This can be taken as a hint that the vessel muscle contains, besides actomyosin, other structure proteins which are responsible for the permanent tension. We have, therefore, in collaboration with Hamoir, investigated extensively vessel muscle extracts by electrophoresis and ultracentrifugation. The results of these investigations will be reported in another communication.

Our findings clearly demonstrate the presence of two structure proteins. The study of their solubility has enabled us to isolate these proteins and to investigate them separately, as well as the actomyosin which was isolated from the vessel muscle.

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Conjugation of *p*-Aminohippurate by the Kidney and Effective Renal Plasma-flow

THE clearance of *p*-aminohippurate has become the standard method for the determination of effective renal plasma-flow, with the assumption that there is no conjugation of *p*-aminohippurate during its passage through the kidney from the blood to the urine. The metabolism of this substance in the body is considered to be similar to that of sulphanilamide which it resembles, namely, acetylation of the *para* amino group. *p*-Acetylaminohippurate and *p*-aminohippurate have similar clearances in the dog and man, so the former may be expected to depress maximal tubular excretion of the latter, but as neither *p*-acetylaminohippurate nor any metabolite in which the *para* amino group is substituted is estimated by the technique used for *p*-aminohippurate, conjugation at sites other than in the kidney should not affect the clearance of the latter. With sulphanilamide, conjugation occurred only in the liver of rabbits and rats, in the liver and spleen in cats, and not at all in the dog (cf. ref. 1). *p*-Aminohippurate itself was not conjugated by rabbit[2] or dog[3] kidney slices *in vitro*, although some conjugation was found with rat[4] and guinea pig[3] kidney slices. From the evidence available, Smith[1] came to the conclusion that conjugation in the kidney did not occur to any significant extent in the species studied (rat, rabbit, cat, dog and man), although it has been suggested by others that conjugation could explain low clearances of *p*-aminohippurate with falling plasma concentrations in man[5].

It will be seen from Table 1 that the conjugation of *p*-aminohippurate does occur to an appreciable extent in the kidney of a number of species; 95 ± 1·3 per cent (22 observations) was recovered after mild acid hydrolysis (100° C. for 30 min., final acid concentration 0·56 *N*). The findings in the rabbit agree with earlier reports, but in the cat only low rates were found in the liver and none demonstrable in the spleen. That this conjugation did interfere significantly with the determination of effective renal plasma-flow was shown in the guinea pig, in which the clearance of free + conjugated *p*-aminohippurate was 1·19 ± 0·05 ($P < 0\cdot01$) (9 determinations) times that of free *p*-aminohippurate on the same samples, with a mean plasma concentration of 0·10 ± 0·015 μmole free *p*-aminohippurate/ml. At this concentration, the calculated values for conjugation and excretion give a value of 1·17 for the above ratio, if the clearance of *p*-aminohippurate is taken to be 13 ml./min. (the mean value of the 9 determinations) and the kidneys weigh 4 gm. each.

The rate of conjugation *in vitro* was maximal at 0·1 μmole/ml., so less error would probably be introduced at higher plasma concentrations, although

Table 1. CONJUGATION OF p-AMINOHIPPURATE *in vitro* BY TISSUE SLICES

Approximately 100 mgm. tissue was incubated for 3 hr. in 2·3 ml. medium containing 30 μmoles sodium acetate in place of an equivalent amount of sodium chloride and 0 4 μmole sodium p-aminohippurate. Values given are means with standard errors with numbers of slices and animals used in brackets

Medium and species	*In vitro* conjugation in μmole/gm. fresh weight/hr.		Estimated *in vivo* excretion of p-aminohippurate by kidney in μmole/gm./hr. at a plasma concentration of 0·1 μmole p-aminohippurate/gm./hr.	Values used for calculation of estimated *in vivo* excretion	
	Liver	Kidney		p-Aminohippurate clearance	Weight of both kidneys
Tris saline (ref. 6)					
Guinea pig		1 31 ± 0 02 (2,1)			
Cross and Taggart medium (ref. 2)					
Guinea pig	0·24 ± 0 01 (2,1)	1·14 ± 0·08 (16,5)	9·8	13 ml./min.	8 gm.
Mouse (white)	0·28 ± 0·03 (2,1)	0 83 ± 0·21 (4,2)			
Rabbit	0·41 ± 0·03 (2,1)	< 0·05 (6,2)	15	2·50 ml./gm. kidney (ref. 1)	
Cat	0·07 ± 0·01 (2,1)	< 0 05 (2,1)			
Cow	0·15 ± 0·01 (2,1)	0 54 ± 0·02 (2,1)	24	4·09 ml./gm. kidney (diodrast) (ref. 7)	
Pig	0·18 ± 0·01 (2,1)	0·35 ± 0·02 (2,1)	20	19·5 ml./kgm. body-weight (ref. 8)	1/175 of body-weight
Sheep	0·42 ± 0 05 (3,2)	0·76 ± 0 04 (3,2)	13	500 ml./min. (ref. 9)	225 gm.

No significant conjugation was seen with slices of spleen, lung or heart muscle of any of the species.

the concentration at which maximal tubular excretion is of importance must be kept in mind. At concentrations less than 0·1 μmole/ml., the rate of *in vitro* conjugation fell with the concentration, so the error introduced would probably be similar to that of about 20 per cent found at 0·1 μmole/ml.

For the species which do conjugate p-aminohippurate in the kidney, a truer value for effective renal plasma-flow would probably be obtained by determining the clearance of free + conjugated p-aminohippurate or of p-acetylaminohippurate alone.

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Enzymatic Reduction of the Yellow Pigment of *Drosophila*

IT has been demonstrated that the enzymatic conversion of dihydrofolic acid to tetrahydrofolic acid plays an important part in various biochemical reactions[1-3]. It has also been suggested that 7,8-dihydropteridine is reduced to the tetrahydro-level by the enzyme extracted from sheep liver in the presence of reduced triphosphopyridine nucleotide[4]. Recently, the yellow pigment (sepiapterin[5]) of *Drosophila* has been confirmed to be 2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine[6]. It was therefore thought that the yellow pigment of *Drosophila* might be also converted to tetrahydropteridine by the enzyme extracted from liver.

The dihydrofolic acid reductase from chicken liver was prepared by Futterman's method[7]. It was found that the reductase attacked 2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine and extinguished both the yellow colour and yellow fluorescence of its solution in the presence of reduced triphosphopyridine nucleotide, but not of reduced diphosphopyridine nucleotide as shown in Fig. 1. The reaction gave a product with a spectrum characteristic of tetrahydropteridine. Thus, this enzyme is capable of reducing the dihydropteridine as well as dihydrofolic acid to 5,6,7,8-tetrahydropteridine with reduced triphosphopyridine nucleotide.

By auto-oxidation, a great portion of the enzymatic reaction product (5,6,7,8-tetrahydropteridine) was converted to a completely dehydrated form having a blue fluorescence, and a small portion was reversed to a partially dehydrated form, namely, the original dihydropteridine, having a yellow fluorescence. The former compound was easily converted to 2-amino-4-hydroxypteridine-6-carboxylic acid by photo-decomposition or oxidation with potassium permanganate. Furthermore, it was decomposed to the same carboxylic acid and aceto-aldehyde by periodate. From these results, it was assumed that the blue fluorescent substance carried the same substituent in the 6-position as that of the original dihydropteridine. Thus, the substance is presumed to be 2-amino-4-hydroxy-6-lactyl-pteridine.

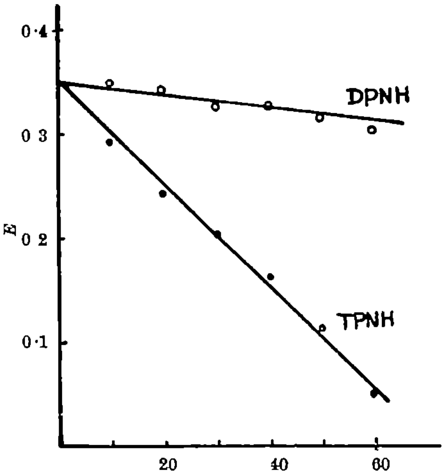

Fig. 1. Effects of reduced tri- and di-phosphopyridine nucleotides on dihydropteridine reductase. Reaction mixture: 10 μgm. 2-amino-4-hydroxy-6-lactyl-7,8-dihydropteridine; 40 μgm. reduced tri- or di-phosphopyridine nucleotide; 45 mM phosphate buffer pH 6·0; 0 3 ml. chicken liver enzyme fraction. Total volume 3 ml. Temperature of incubation 37° C. A spectrum characteristic of the dihydropteridine at 410 mμ

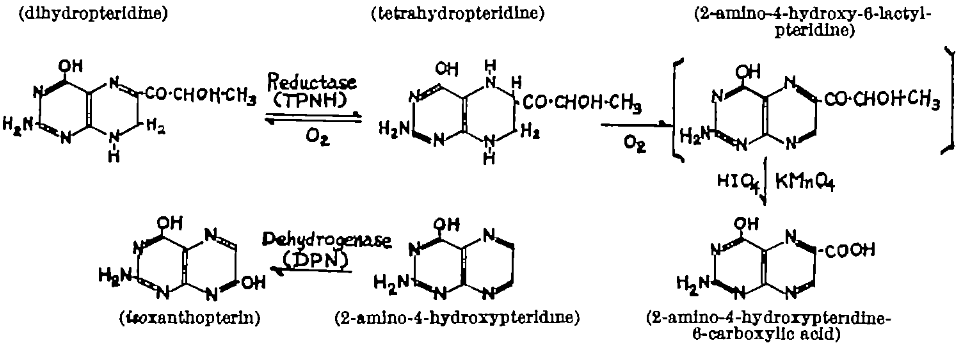

Fig. 2. A possible metabolic pathway of pteridines in *Drosophila*

The product in the enzymatic reaction mixture contained a considerable amount of *iso*xanthopterin that was not present in the original reaction mixture. I confirmed that the chicken liver extract was capable of converting 2-amino-4-hydroxypteridine to *iso*-xanthopterin. Therefore, it is suggested that 2-amino-4-hydroxypteridine was derived from the decomposition of tetrahydropteridine and converted enzymatically to *iso*xanthopterin.

In the presence of reduced triphosphopyridine nucleotide, the partially purified enzyme of *Drosophila* was capable of reducing the dihydropteridine to the tetrahydropteridine from which 2-amino-4-hydroxy-tryptamine can be derived by auto-oxidation. It has been shown that the latter can be converted to *iso*xanthopterin by pteridine oxidase[8] or dehydrogenase[9] extracted from *Drosophila*. These observations suggest a metabolic pathway of pteridines in *Drosophila* such as is shown in Fig. 2.

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Cardiac Effects of Streptolysin O in Rabbits

Although several of the known streptococcal enzymes or toxins have been suspected of playing a part in the pathogenesis of the heart lesions in rheumatic fever, a number of reports have suggested that streptolysin *O* may be of some importance in the causation of focal cardiac damage in experimental animals. Kellner *et al.*[1] have demonstrated that the rabbit heart *in vitro* is extremely sensitive to this substance. Schwab *et al.*[2], among others, showed that rabbits pre-treated with certain streptococcal factors developed a high incidence of focal cardiac lesions on subsequent injection of streptolysin *O* containing filtrates. These investigations were carried out with impure preparations of unknown complexity.

Previous reports from this laboratory have described the value of agar precipitin analysis in the study of human streptococcal infections[3-4]. In the course of this work, very highly purified streptolysin *O* preparations were obtained, using a combination of continuous-flow electrophoresis and column chromatography on calcium phosphate (hydroxylapatite) gels. Sufficient amounts of similar preparations from a group *A* and group *C* streptococcal strain have been separated in order to study their effects on intravenous injection in rabbits. Such observations were made in an attempt to translate the above-cited findings to the *in vivo* environment. Although the final fractions were not as free of contamination immunologically as those obtained previously[5], they were both extremely potent. The group *A* fraction revealed about 160,000 hæmolytic units/mgm. protein, and the group *C* about 60,000 units/mgm. Both contained small amounts of diphosphopyridine nucleotidase, and the group *A* showed traces of a third component immunologically. The group *C* material, only, contained appreciable amounts of apparently non-protein substances in the lyophilized powder (about 40 per cent).

The cardiotoxic effects of intravenously injected streptolysin *O* in rabbits were studied electrocardiographically in all instances. In a number of cases, femoral artery blood pressures and electroencephalograms were also recorded. Albino rabbits from a single dealer (2·6–3·2 kgm.) of either sex were used. The streptolysin solutions, and all others, were made in 0·1 *M* sodium phosphate at *p*H 7·2. Activation of the streptolysin was carried out by adding a neutralized 1-cysteine hydrochloride solution to a final concentration of 10 mgm./ml., and incubating at 30° C. for 10 min., following which it was kept in an ice-bath.

The *LD*50 of the group *A* activated (reduced) streptolysin was approximately 0·15 mgm. dry weight of the lyophilized powder, while that of the group *C* material was 0·75 mgm. dry weight (about 0·45 mgm. protein). A total of 50 rabbits was used for these assays.

Following injection, with few exceptions, death ensued within 5 min., or less, associated with convulsions and respiratory standstill. It was observed that considerable electrocardiographic changes always preceded the convulsions, and could be produced within as short a time as 3 sec. with 3–4 *LD*50. The injection itself took approximately 2–3 sec. Examples of the very rapid electrocardiographic changes are shown in Figs. 1*a* and *b*. There was immediate transition from normal sinus rhythm to ventricular arrhythmia, followed by electrocardiographic silence.

With smaller but still lethal doses (1–2 *LD*50) the reaction often took somewhat longer to develop. An

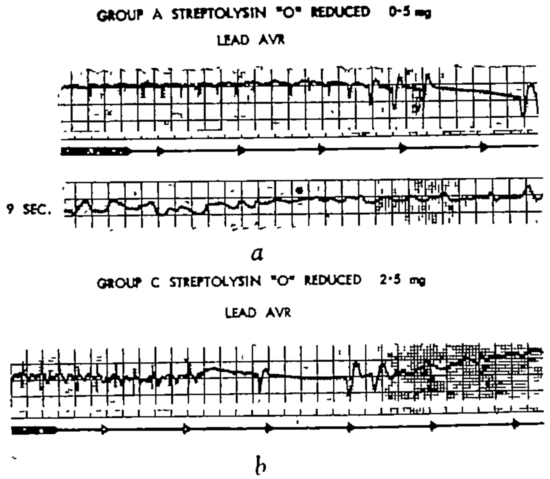
GROUP A STREPTOLYSIN "O" REDUCED 0·5 mg
LEAD AVR
9 SEC.
a
GROUP C STREPTOLYSIN "O" REDUCED 2·5 mg
LEAD AVR
b

Fig. 1. Electrocardiographic tracings of rabbits receiving intravenous injections of reduced activated streptolysin *O* in several multiples of the *LD*50 dose. The injection period is marked on the 1-sec. time interval record. *a*, Group *A* streptolysin *O*, 0·5 mgm. dry weight/0 5 ml. (Lower strip tracing starts 9 sec after the injection was completed.) *b*, Group *C* streptolysin *O*, 2·5 mgm. dry weight/1 0 ml.

example of the electrocardiographic changes occurring in a rabbit receiving such a dose of the group *A* preparation is shown in Fig. 2. Within 25 sec., unifocal ventricular rhythm with ST segment depression occurred. Within 125 sec., typical ventricular arrhythmia was seen, and within 224 sec., ventricular tachycardia was soon followed by ventricular fibrillation. In all instances, the motor convulsions occurred without electroencephalographic convulsive discharges.

In nine of thirteen cases, non-lethal doses of the fractions produced temporary electrocardiographic changes, such as ST—T depression, supraventricular or ventricular extrasystoles, or bigeminal rhythm, but these were erratic in occurrence. Sometimes they lasted intermittently at least as long as 18 hr.

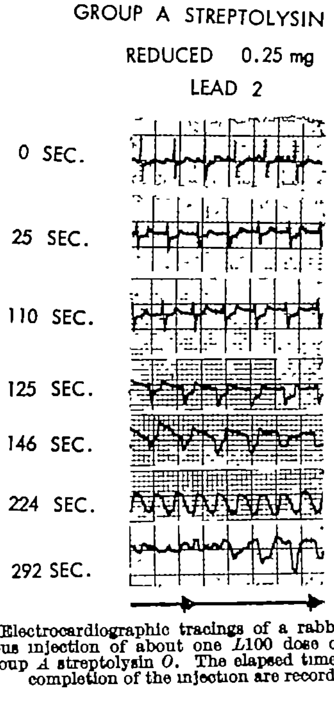

Fig. 2. Electrocardiographic tracings of a rabbit receiving an intravenous injection of about one *L*100 dose of reduced activated group *A* streptolysin *O*. The elapsed times following the completion of the injection are recorded

When lethal doses were given, the blood pressure dropped rapidly to less than 25 mm. mercury. This fall occurred either following or prior to the electrocardiographic changes, and was later followed by electroencephalographic silence.

Evidence that the lethal effects were due to streptolysin *O* was obtained in several ways, in addition to the high degree of apparent purification immunologically. Much larger doses of the reversibly oxidized inactive form were required to bring about lethal effects, 1 mgm. dry weight of the group *A*, and 6·5 mgm. of the group *C* preparations being required. The time required for death was substantially longer with the oxidized form as compared to equivalent amounts of the reduced. It is likely that the streptolysin may be gradually reduced to activity *in vivo* in view of the known oxidation-reduction potential of tissues[6].

Cholesterol, a potent inhibitor of the hæmolytic activity of streptolysin *O* (ref. 7), protected rabbits against the lethal and electrocardiographic effects, when an aqueous suspension was mixed with the challenge dose. Diphosphopyridine nucleotidase has been shown to be unaffected by cholesterol, and by reduction with cysteine[8].

Numerous control experiments with cysteine, and different streptococcal fractions previously isolated[5], including deoxyribonuclease *B*, streptococcal proteinase, C-carbohydrate protein complex[9], and an antigen possibly related to scarlet fever toxin, failed to produce similar electrocardiographic effects. *Shigella paradysenteriae* Flexner type III endotoxin also did not show any such reactions.

These observations showed that streptolysin *O* from a group *A* and a group *C* streptococcal strain may kill rabbits extremely rapidly when given intravenously. Extensive electrocardiographic and blood pressure changes were observed preceding death. With several multiples of the lethal dose, cessation of cardiac activity was noted within 3–5 sec. following the injection. The findings presented here support the speculation that streptolysin *O* may be the factor causing cardiac lesions *in vivo*, as a result of repeated streptococcal infections in experimental animals (for example, ref. 10). Their relation to the pathogenesis of human rheumatic fever remains to be determined.

These investigations were supported by research grants from the Office of Naval Research, the Life Insurance Medical Research Fund, and the New York Heart Association.

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Levels of Serotonin during Convulsion and the Effect of Reserpine

IN view of the contradictory reports[1-3] with respect to changes in the 5-hydroxytryptamine (5-HT) levels of the mammalian central nervous system during convulsion, this problem was re-examined in rats in which convulsion was induced by intraperitoneal injection of fluoroacetate or 'Metrazol'. The former convulsant was particularly preferred because, unlike the convulsive agents generally employed, its action can be related to specific biochemical events, that is, blocking of the citric acid cycle[4] with concomitant changes in certain amino-acid[5]- and ammonia[6]-levels of the central nervous system.

The serotonin content of brain, small intestine and spleen of rats treated with convulsants, with or without simultaneous administration of reserpine, was compared with control animals. The solution of reserpine for intraperitoneal injection was prepared by dissolving reserpine crystals (100 mgm.) in 0·1 ml. of concentrated formic acid, 2·5 ml. propylene glycol, 2·5 ml. absolute alcohol and water to make the volume 20 ml. (Formate was used instead of glacial acetic acid to avoid the competition of acetate with fluoroacetate.) The animals which did not receive reserpine received only intraperitoneal injection of reserpine solvent. The animals were killed at 60 min., with the onset of convulsion at 30 min. The amount of fluoroacetate injected was greater than the *LD*50, 5 mgm./kgm. for rats, in order to ensure the severity and the recurrence of seizures between onset and time of killing.

Statistical analysis of the values in Table 1 indicates that fluoroacetate-produced convulsion does not cause an increase of serotonin-level in the brain or in the other organs analysed. Administration of reserpine did not produce any delay in the onset of convulsions or in the incidence of seizures. However, it depleted serotonin within 1 hr. by 78 per cent from the brain of convulsing animals, but not from the spleen or the intestine, a pattern similar to that produced by reserpine in non-convulsing rats[8]. Administration of reserpine did not alter the fluoroacetate-produced citrate accumulation in the brain to any degree of statistical significance. In another series of experiments 'Metrazol' (60 mgm./kgm.) was administered intraperitoneally alone and with reserpine. 'Metrazol', like fluoroacetate, had no effect on the 5-HT values of the brain of convulsing rats killed 1 hr. after administration of the drug. Again, convulsions produced by 'Metrazol' had no effect on the release of 5-HT by reserpine from the brain.

Table 1. EFFECT OF CONVULSION ON 5-HYDROXYTRYPTAMINE CONTENT OF SOME RAT TISSUES AND THE INFLUENCE OF RESERPINE. Sprague–Dawley male rats (350–400 gm.) received intraperitoneally sodium fluoroacetate (8 mgm./kgm.) and reserpine (6 mgm./kgm.) as indicated. 5-Hydroxytryptamine was determined by the spectrophotofluorometric method (ref. 7) and the citrate, as described elsewhere (ref. 4). Numbers of animals indicated in brackets

Group	5-Hydroxytryptamine (μgm./gm. wet tissue) Brain	Spleen	Small intestine	Citrate (μgm./gm. wet tissue) Brain
Control (10)	0·41 ± 0·03*	2·02 ± 0·22	2·08 ± 0·30	19 ± 1·3
Fluoroacetate (10)	0·48 ± 0·03	1·78 ± 0·27	2·11 ± 0·36	151 ± 10
Fluoroacetate + reserpine (10)	0·11 ± 0·05	1·90 ± 0·28	2·30 ± 0·30	119 ± 13
Reserpine (5)	0·12 ± 0·05	2·34 ± 0·67	3·00 ± 0·39	
'Metrazol' (6)	0·40 ± 0·02	2·17 ± 0·18	3·43 ± 0·31	
'Metrazol' + reserpine (6)	0·25 ± 0·05	2·40 ± 0·25	3·16 ± 0·26	

* *S.E.*

It can safely be stated that there is no statistically proved evidence to demonstrate that fluoroacetate, and, under the conditions of our experiments, 'Metrazol', have any effect on the 5-HT levels of rat brain.

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Enzymic Release of Sialic Acid and Inactivation of Human Menopausal Gonadotropin

IT has been shown that the biological activity of chorionic and pituitary gonadotropins is lost when the hormone materials are treated with influenza virus or with receptor-destroying enzyme[1-3]. This enzyme has been recognized as neuraminidase[4], and the loss of biological activity of gonadotropins on treatment with the above catalytic agents was accompanied by the release of sialic acid. It seemed desirable to investigate the effect of neuraminidase on the chemical composition and biological activity of a purified preparation of human menopausal gonadotropin[5,6].

The relative potency of the preparation used, in terms of human menopausal gonadotropin-24, was 100 when tested by the augmentation of uterus weight in 3-week-old female mice. It contained 7·8 per cent of sialic acid, determined by the diphenylamine reaction[7] and expressed as N-acetylneuraminic acid. Heating at 100° C., *p*H 2, for 1 hr. destroys the activity and quantitatively removes sialic acid, but it is possible that other bonds (sugar, amino-acid) were broken.

10 mgm. of human menopausal gonadotropin preparation, dissolved in 10 ml. *M*/15 phosphate buffer, *p*H 6·3, was incubated with about 30,000 units of neuraminidase (receptor-destroying enzyme, Behringwerke) at 35° C. After 24 hr. the solution showed a loss of gonadotropic activity of 80 per cent, as compared with a control kept under the same conditions but without the receptor-destroying enzyme. Reaction mixture was then dialysed twice against 10 vol. of water at 0° for 48 hr. About 60 per cent of initial sialic acid was found in the dialysate. After concentration, the dialysate was applied to a column of 'Dowex 2' 200–400 mesh (20 × 0·8 cm.), equilibrated in 0·1 *M* formic acid[8]. The effluent was collected in 3-ml. fractions which were analysed for their sialic acid content. Elution with 0·3 *M* formic acid yields two peaks (fractions 13–19 and 24–29).

Paper chromatography (*n*-butanol / pyridine / 0·1*N* hydrochloric acid, 5 : 3 : 2) showed that the two peaks contained the same component which was identified as N-glycolylneuraminic acid. Release of glycolic acid, after hydrolysis by *N* sulphuric acid, confirmed this result[9].

Although the human menopausal gonadotropin preparation was not pure, the following conclusions may be drawn from the experimental results: (*a*) human menopausal gonadotropin contains sialic residues which are accessible to neuraminidase; (*b*) enzymic release of the sialic acid residues reduces the biological activity of human menopausal gonadotropin by 80 per cent or more. Bound sialic acid is an essential part of the molecule.

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Seasonal Variation in Blood Pressure in Man

SEVERAL of man's bodily functions, such as thyroid activity, are known to show a characteristic seasonal pattern; but in general such seasonal variations have received less attention in man than in many other animals. In particular, seasonal trends in blood pressure do not seem to have been measured hitherto.

The data presented here were derived from measurements on 56 middle-aged men who had been observed for 1–3 years at a clinic for ischæmic heart disease. Most had suffered cardiac infarction, but for all of them at least two months had elapsed since the acute illness and during the period of observation they were in a clinically stable state. There were three observers, but as a rule each subject's blood-pressure measurements had all been taken by the same observer. All measurements were made in a comfortably warm room after a period of rest and using standard instruments.

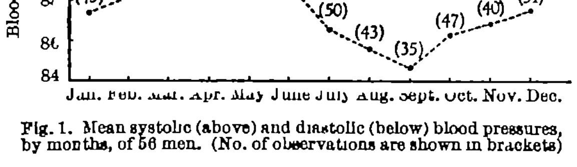

Fig. 1. Mean systolic (above) and diastolic (below) blood pressures, by months, of 56 men. (No. of observations are shown in brackets)

The results are shown in Fig. 1, where mean systolic and diastolic pressures have been plotted by months; the technique of grouped means has been followed; the value at each month represents the mean of the values for that and the two adjacent months. There is a clear seasonal trend, with a peak covering the spring and a trough in the late summer.

This pattern is quite different from that of ischæmic heart disease morbidity and mortality, which follow the reciprocal of air temperature, namely, a peak in January and February and a lower level throughout the late spring and summer.

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Relationship between Urinary Hyaluronidase and Diuresis

In a recent communication, Berlyne[1] states that he was unable to corroborate my results[2] on the relationship between the hyaluronidase activity of the urine and diuresis.

(1) Berlyne believes that my method is unsuitable for quantitative assay of urinary hyaluronidase, as I did not take into account the drop of substrate viscosity before the first reading. But the determination of enzyme activity by the method of Swyer and Emmes[3], used by me, does not depend on the initial substrate viscosity. The modified method of McLean and Hale[4], used by Berlyne, has no advantage over mine.

He thinks that my results are not precise enough because I did not take into account the electrolyte effect. But in practice I did take it into consideration, and some of my experiments were performed on samples of urine previously dialysed. On the removal of electrolytes, hyaluronidase activity somewhat increases. But the correction introduced by dialysis does not alter the main relationship and curves of dialysed urine, as well as of electrolyte-containing urine run in parallel[5].

(2) Berlyne believes that I was wrong when giving the results as the fermentation activity of the urine in arbitrary units. In his opinion, the results should be expressed by the amount of the enzyme excreted per minute. I cannot agree with this.

The urinary hyaluronidase is produced by renal cell secretion under the action of antidiuretic hormone. The enzyme performs a specific function in the kidney. It depolymerizes hyaluronic structures of the tubules, and this provides the conditions for antidiuretic reaction. The appearance of hyaluronidase activity in urine should not be regarded as a process of excretion. This phenomenon is merely a side-effect of the antidiuretic hormone enabling one to verify the mechanism of the hormone action.

From the above point of view, there is no reason to express the results as the product of the enzyme concentration (irrespectively of any units) by the volume of urine, as it is done for estimating the excretion of substances removed by the kidney. As this operation has no physiological meaning in this case, it becomes purely arithmetical and completely distorts the essence of the phenomenon under study.

(3) The probability of my physiological interpretation of the phenomenon in question was investigated in two series of experiments. In one (jointly

with V. Vasiljeva and J. Natochin), dogs were decerebrated, the cut running behind the tuber cinereum. The hypophysis was removed and the kidney denervated. To maintain a steady diuresis-level, mannitol solution was administered intravenously. A few urine samples were collected for 1 hr. Then a large dose of 'Pitressin' was injected, and urine was collected for another hour.

On the removal of antidiuretic hormone-producing structures, urine, unlike normal dog urine, does not exhibit hyaluronidase activity during osmotic diuresis. Under the hormone action, the enzyme appeared in urine in spite of the fact that urine flow increased.

In another series of experiments (jointly with L. Ivanova) on dogs with a permanent fistula of the ureter, the animals were deprived of water for 24 hr. Urine collected during hydropeny revealed high hyaluronidase activity. The second urine sample was collected after the dog was allowed to drink water and then diuresis exceeded 1 ml. per min. In this sample no enzyme was found. Then urine was concentrated in vacuum. Thus it was artificially reduced to the volume corresponding to the rate of natural urine flow during dehydration. Yet even then the urine did not acquire hyaluronidase activity. Thus the loss of enzyme at water diuresis cannot be accounted for by the dilution alone, though this factor certainly exercises its usual effect.

(4) The difference between osmotic and water diuresis is particularly distinct if the corresponding correction eliminates the dilution effect. I did this in the following way. Urine samples collected at different levels of diuresis were dialysed and then concentrated in vacuum to the volumes corresponding to a urine flow of 0·2 ml./min. In this way I obtained the results shown in Fig. 2 of my communication in *Nature* to which Berlyne refers. All the points on the curve, both for water and osmotic diuresis, represent urine activity under reduced conditions. Hence the results are not complicated either by dilution or by electrolyte effect. Considering the process under such constant conditions, I arrived at the conclusion that secretion of hyaluronidase by renal cells decreases during water diuresis, but it remains at a constant level during osmotic diuresis.

By direct estimation of hyaluronidase activity of urine collected during osmotic diuresis without any correction for dilution, I certainly found that activity drops as the flow of urine increases.

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I AM grateful for the opportunity afforded by Prof. Ginetzinsky's letter to clarify certain points in my previous communication[1] which I had omitted as they are dealt with at length elsewhere[2]. I hope to resolve some of the problems mentioned by Prof. Ginetzinsky point by point.

(1) It has been shown by McLean and Hale[3] and confirmed by me[1,2] that the ionic strength of the hyaluronic acid – buffer – hyaluronidase system must be kept constant if quantitative measurement of hyaluronidase is to be achieved. In a system containing constant amounts of hyaluronidase and hyaluronic acid, as the ionic strength of the medium is increased from 0 to 0·3 *M* the apparent hyaluronidase activity changes from 0 to 90 units (Fig. 3 of ref. 3). Thus it is essential to control electrolyte concentration carefully if quantitative results are to be obtained with urine. I chose to keep ionic strength constant by electrometric estimation and addition of salt in appropriate amounts rather than use dialysis, where adsorption of hyaluronidase might take place on to the dialysing membrane. The method used is published in detail elsewhere[2].

(2) It seems reasonable to express urinary hyaluronidase findings in terms of excretion per unit time, as is the practice with other urinary solutes. If one accepts the statement that hyaluronidase appears in the urine "merely as a side-effect of the antidiuretic hormone" and "not as a process of excretion" in order to justify the use of hyaluronidase concentration as opposed to excretion per minute, then there is little point in measuring hyaluronidase excretion (either per ml. or per min.) as evidence for or against Ginetzinsky's hypothesis of the role of hyaluronidase in the urine-concentrating mechanism. If one plots urinary hyaluronidase concentration against urine flow, one obtains a similar graph to that which would be obtained by plotting creatinine concentration against urine flow; that is, the lower the flow, the higher is the concentration of both hyaluronidase and creatinine. Nobody would suggest that creatinine is implicated in the antidiuretic hormone mechanism of urine concentration, yet Ginetzinsky used evidence of this type to suggest that hyaluronidase is instrumental in the antidiuretic hormone mechanism for urine concentration.

(3) I am not familiar with the decerebrate hypophysectomized dog preparation and am unable to comment on this experiment without knowing details of urine electrolyte concentration, urine flow-rate, etc. In Ginetzinsky's article (ref. 4, Fig. 2) he shows that the dog in osmotic diuresis in four out of five results has urine flows of 1·5 ml./m.^{2}/min. or less. These are very small for adequate mannitol osmotic diuresis, and are not strictly comparable with my results in humans, where urine flows up to 9·5 ml./min. were reached.

The fact that Prof. Ginetzinsky in only some experiments dialysed and then reduced to constant volume indicates that he realized the need for measuring hyaluronidase excretion per min. Unfortunately, he did not do this in all experiments; hence his results are not uniformly expressed and therefore direct comparisons cannot be made.

Since my initial communication I have extended the number of observations on humans in water

Table 1

Urine (ml./min.)	Osmolality of urine (mOsmols/kgm.)	Hyaluronidase (units/ml.)	Hyaluronidase excretion per min.
3·42	240	0 6	2·04
4·2	133	0 25	1 1
4 75	154	0·45	2 1
5 6	162	0·38	2·1
6 23	91	0·75	4·7
6·7	104	0·45	3 0
7·8	94	0·125	0·98
8·06	103	0·26	2·1
9·55	84	0 12	1·2
10·7	55	0 12	1·3

diuresis in the range 3·42–10·7 ml./min., as shown in Table 1.

It will be seen that I found detectable amounts of hyaluronidase even at high urine flows in water diuresis whereas Ginetzinsky does not. The most obvious explanation for this discrepancy is that I was using a more sensitive method, and one which does not involve loss from dialysis.

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HISTOCHEMISTRY

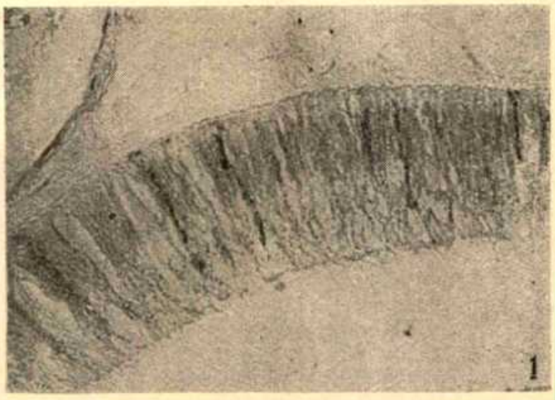
Presence of Enterochromaffin Cells in the Gut of Amphioxus

Histochemical evidence has been found for the presence in the gut of amphioxus of argentaffin cells, which do not appear to have been described formerly; because of their localization and their histochemical characteristics, they can be considered to be of enterochromaffin type, shown to be present in other groups of Chordata (by Vialli and Erspamer[1] for all vertebrates, except Cyclostomata and Teleostea, and by Lison[2] for Ascidians).

The histochemical characteristics of typical enterochromaffin cells are well established[1], namely, positive reaction in the chromaffin, argentaffin and diazonium in alkaline solution tests. Other staining methods (non-histochemical) are also suitable for detection of enterochromaffin cells, based on their acidophilia and argyrophila. Moreover, Jacobson[3] observed later that the specific substance fixed in the granules of enterochromaffin cells is removed by a hot pyridine–methanol mixture. Finally, quite recently, Pearse in his book[4] presents as characteristic of the enterochromaffin cells five major histochemical tests (chromaffin, argentaffin, diazonium, indophenol and Gibbs's reactions) and other subsidiary ones, including the autofluorescence, the Schmorl ferric–ferricyanide reaction and the Pearse thioindoxyl reaction.

For the present work, four specimens of *Branchiostoma lanceolatum* Pallas, 30–50 mm. long, after having been fixed in 10 per cent formol-saline and embedded in paraffin, were cut transversely to provide a series of sections of 6μ thickness.

In the gut, some cells were found to be strongly positive by the Masson–Hamperl argentaffin reaction. The argyrophil methods (particularly the Bodian reaction) gave also positive results, corresponding to those of the former reaction as regards number and localization of the cells. Positive results were obtained, however, with very faint contrast compared to the background, after the chromaffin reaction, the Gibbs indophenol reaction, the Lillie diazo–safranine reaction at *p*H 9·4 and the Schmorl ferric–ferricyanide reaction. No positive reaction was found with other diazo-coupling methods (with sulphanilic acid and stabilized diazonium salts), the Pearse thioindoxyl reaction or autofluorescence; after treatment with a pyridine–methanol mixture at 60° C. for five days, the sections did not stain with any of the methods mentioned above.



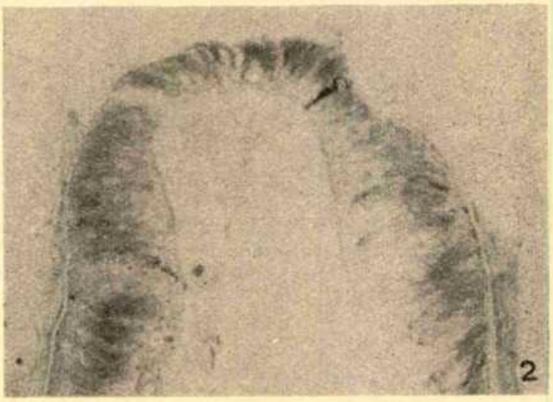


Fig. 1. Left wall of mid-gut; Masson argentaffin reaction. Epithelial ciliated cells are closely packed; some narrow enterochromaffin cells are seen. (× 500)

Fig. 2. Dorsal groove of hind-gut; Masson argentaffin reaction. Epithelial ciliated cells are less packed and narrow: one enterochromaffin cell is clear, but others are seen only with few granules in focus

The cells under examination are very slender in shape, occupying the whole height of the intestinal epithelium, and are slightly larger at the base, where the specific granules may be more evident. These cells are usually isolated, and not more than 5–8 occur in each section.

The enterochromaffin cells do not occur in the anterior tract of the digestive tube; they occur in the posterior part of the mid-gut on the left wall and the dorsal zone, to continue only dorsally in the ilio-colon ring and in the hind-gut. They are localized in the strongly ciliated tracts, that is, in the lateral ciliated tract of the posterior part of the mid-gut and the dorsal ciliated tract in the ilio-colon ring and in the hind-gut (the terminology corresponds with that of Barrington[5]). This peculiar distribution requires further study in connexion with the morphogenetic and functional interpretation.

I have not been able to find evidence for all the ordinary histochemical characteristics already mentioned for enterochromaffin cells of vertebrates, in which the presence of 5-hydroxytryptamine (enteramine) has been proved[6,7]; nevertheless, the failure of some tests may depend on decreased sensibility of the reactions, as has been confirmed in other cases, or on decreased contrast of the stain compared with the background. This problem was recently discussed by Vialli[8] in connexion with staining methods for enterochromaffin cells.

A more extensive investigation of this kind on which I am engaged should contribute to a unified interpretation regarding the biological meaning of the enterochromaffin system and the metabolism of 5-hydroxytryptamine, at least within the Chordata.

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HÆMATOLOGY

Fibrinolytic Activities present in Human Blood Platelets

BLOOD platelets have been shown to contain antiplasmin activity[1-3]. The experiments to be described in this communication demonstrate that blood platelets interfere in still another way in fibrinolysis. The fibrinolytic activity brought about by streptokinase comprises two steps[4]: (1) activation of a proactivator which could be identical in man with plasminogen; (2) transformation of plasminogen to plasmin by the activator, resulting from reaction (1).

When human blood platelets are added in increasing concentrations to a test system composed of streptokinase and bovine fibrinogen, clotted by addition of thrombin–calcium solution, a progressive increase of fibrinolytic activity is observed. This was demonstrated by each of the following three methods: estimation of clot lysis-times, thrombelastographic recordings and the fibrin plate method according to Astrup and Müllertz[5]. Table 1 represents the results obtained with clot lysis-time determination.

Table 1. INFLUENCE OF HUMAN BLOOD PLATELETS ON STREPTOKINASE-INDUCED LYSIS OF BOVINE FIBRIN

Concentration of blood platelets in the added suspension	Clot lysis time
5,000,000/mm.^{3}	15 min.
2,500,000/mm.^{3}	21 min.
1,000,000/mm.^{3}	40 min.
500,000/mm.^{3}	85 min.
100,000/mm.^{3}	18 hr.
Isotonic sodium chloride (control)	more than 48 hr.

0·1 ml. blood platelets suspension is mixed with 0·1 ml. streptokinase solution (1,000 U./ml.) and added to 0·5 ml. bovine fibrinogen (1 gm. per cent). The mixture is clotted by addition of 0·2 ml. Thrombin 20 U./ml., dissolved in *M*/20 calcium chloride. Determination of clot lysis-time at 37° C.

The bovine fibrinogen ('Rinderfibrinogen' from Behring-Werke, Marburg/Lahn, Germany) used as substrate contained large quantities of plasminogen but was nearly free of proactivator activity. In control experiments where blood platelets were substituted by isotonic sodium chloride solution, lysis did not occur within a 24-hr. period. The thrombin ('Test Thrombin' from Behring-Werke) used was found to be void of any appreciable contamination with plasminogen or proactivator. The streptokinase was a highly purified preparation, kindly supplied by Behring-Werke. Veronal buffer (*p*H: 7·35, μ: 0·154) according to Owren, was used for dissolving fibrinogen, streptokinase, urokinase and plasmin. Blood platelets were suspended in isotonic sodium chloride solution.

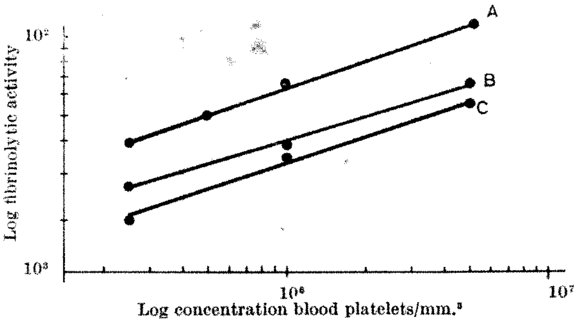

Fig. 1. Influence of washing platelets on their promoting effect on streptokinase-induced lysis of bovine standard fibrin. Results of determinations of fibrinolytic activity by the fibrin plate method. Double logarithmic graph. Abscissa: concentration of blood platelet suspension tested; ordinate: fibrinolytic activity as measured by the product of the two diameters of the lysed zones. The tested platelet suspension is mixed with an equal volume of streptokinase solution (5,000 U./ml.); 0·03 ml. of this mixture is placed on the surface of the plate. Curve *A*, blood platelets washed once with isotonic sodium chloride solution; curve *B*, platelets washed 5 times; curve *C*, platelets washed 10 times

The blood platelets tested in our experiments had been prepared by several techniques. Successively, lyophilized human blood platelets[6] and human blood platelets isolated from sequestrene, citrated or A.C.D. plasma were used. They all had a similar enhancing effect on streptokinase-activated lysis of bovine fibrin.

In contrast to the influence on streptokinase-induced lysis of bovine fibrin, human blood platelets had a clearly inhibiting effect on urokinase (kindly supplied by Dr. J. Ploug, Leo Pharmaceuticals, Ballerup, Denmark) or plasmin (the chloroform-activated bovine fibrinolysin, kindly supplied by Dr. E. Loomis, Parke Davis and Co., Detroit) activated lysis.

Further work showed that washing platelets resulted in a gradual weakening of the promoting effect on streptokinase-activated fibrinolysis. After ten washings, however, there was still an appreciable residual activity (Fig. 1). The antiplasmin effect, on the other hand, was only slightly diminished by the washing procedures.

Heating of platelets at 80° C. for 15 min. completely destroyed the above-described lysis-promoting activity. Instead of an enhancing an inhibiting effect on streptokinase-induced lysis was observed (Table 2). The antiplasmin activity was not diminished by this heating procedure.

Our results, which will be published in detail elsewhere, demonstrate that blood platelets, on addition of streptokinase, form an activator of plasminogen. This can be explained by the assumption that blood platelets contain so-called proactivator activity. The presence of plasminogen in blood platelets seemed to be ruled out as a possible explanation for the observed facts. Indeed, no fibrinolytic activity could be produced when mixtures of blood platelets and streptokinase were tested on heated fibrin plates, in which the plasminogen had been destroyed.

Table 2. INFLUENCE OF HEATING PLATELETS ON STREPTOKINASE-INDUCED LYSIS OF BOVINE FIBRIN

Concentration of lyophilized platelets in the added suspension	Clot lysis-time	
	Unheated platelets	Heated platelets (15 min. at 80° C.)
10 mgm./ml.	8 min.	120 hr.
2·5 mgm./ml.	18 ,,	72 ,,
0·625 mgm./ml.	42 ,,	46 ,,
Isotonic sodium chloride (control)	39 hr.	39 ,,

0·1 ml. lyophilized human blood platelets are mixed with 0·1 ml. streptokinase solution (5,000 U./ml.) and added to 0·3 ml. bovine fibrinogen (2 gm. per cent). This mixture is clotted by addition of 0·1 ml. thrombin (20 U./ml.) dissolved in *M*/10 calcium chloride. Determination of clot lysis-time at 37° C.

The investigation of the influence of washing and heating showed that the platelet proactivator is to be distinguished from the blood platelet antifibrinolysin. These two activities cannot be caused by an identical platelet substance. The lysis-promoting property of blood platelets completely masks their antifibrinolytic effect in the case of streptokinase-activated lysis. Whether the proactivator activity of blood platelets is to be attributed to an intrinsic or adsorbed platelet component cannot be stated with certainty at present. In any event, ten-fold washing of blood platelets does not extinguish its effect.

These observations were confirmed in relation to fibrinogenolysis by determination of the so-called antithrombin VI activity[7], arising in a system composed of bovine fibrinogen, streptokinase and blood platelets. It was established that the apparent amount of antithrombin VI was directly proportional to the concentration of blood platelets present in the test system.

It seems highly probable that the proactivator activity of human blood platelets plays an important part in the spontaneous fibrinolytic activity of human blood. Indeed, the latter is known to be chiefly caused by an activator of plasminogen, probably formed by activation of proactivator[8].

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Precipitating Factor for Aggregated γ-Globulins in Normal Human Sera

SERA of patients with rheumatoid arthritis often form a precipitate when mixed with aggregated γ-globulins[1]. The precipitate consists of a 19 *S* γ-globulin ('rheumatoid factor'), variable amounts of 7 *S* γ-globulin and more rapidly sedimenting aggregates of γ-globulin[2]. The latter are known to be anti-complementary[3]. While studying a test for rheumatoid arthritis[4], it was found that normal human sera also form a precipitate with aggregated γ-globulins, although more slowly and less abundantly.

Aggregated γ-globulins were prepared by treating 1 per cent solutions of Cohn's human fraction II in 1/15 molar phosphate buffer, *p*H 7, containing 1 : 10,000 merthiolate, with 0·25 per cent formalin for two weeks at room temperature, followed by dialysis in the cold against the same buffer without formalin. This resulted in marked opalescence of the solutions. Ultracentrifugal analysis showed the presence of large aggregates. When mixed in capillary tubes with an approximately equal amount of human serum, these preparations yielded a precipitate after incubation for 2 hr. at 37° C. and for 18 hr. at 4° C. Sera of 200 patients, aged 5–80, with various diseases, and of 80 adolescent and adult healthy subjects, were found to react. Exceptions were found only in the sera of the 10 new-born babies tested, which showed no, or minimal, precipitate, although their mothers' sera formed a heavy precipitate.

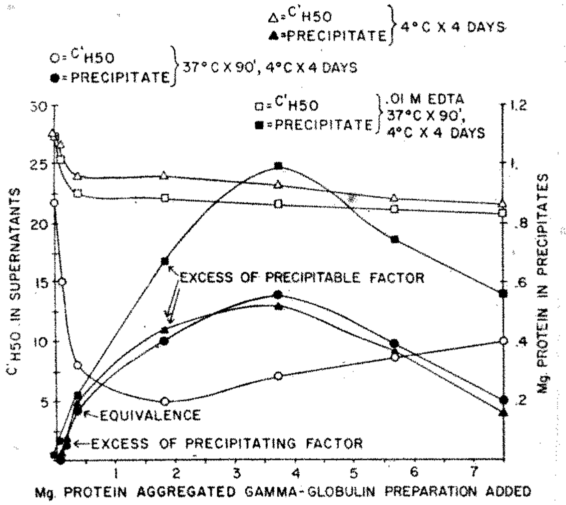

Fig. 1. Precipitating reaction between equal aliquots of normal human serum (1 ml.) and increasing amounts of aggregated γ-globulins: determination of precipitate protein, and of hæmolytic complement titre in the supernatants

Quantitative tests showed that the precipitation reaction could be inhibited by an excess of aggregated γ-globulins (Fig. 1). The supernatants were tested for presence of the precipitating serum factor (by mixing them with aggregated γ-globulins) and for the precipitable factor of aggregated γ-globulins (by mixing them with normal human serum). Three zones were thus found, one with excess precipitating factor, another with neither precipitating nor precipitable factor (equivalence), and a third with only precipitable factor in the supernatants. The amount of precipitate continued to increase beyond the equivalence zone, as is also observed in the precipitation reaction between rheumatoid serum and aggregated γ-globulins[5], as well as in classical antigen–antibody reactions, when the antigen is aggregated[6]. The amount of precipitate recovered from each tube usually failed to exceed the amount of aggregated γ-globulins added to the serum, which suggests that only a fraction of the preparation added takes part in the formation of the precipitate.

Decomplementation of the serum by absorption with 1·2 mgm. of heterologous antigen–antibody precipitates (bovine serum albumin–rabbit anti-bovine serum albumin, or egg albumin–rabbit anti-egg albumin) per ml. of serum prevented subsequent precipitation with aggregated γ-globulins.

Conversely (Fig. 1), the supernatants obtained after incubation for 90 min. at 37° C. and for 4 days at 4° C. showed a decrease in the titre of complement as measured in 50 per cent hæmolytic units. This 'anti-complementary' effect increased with increasing amounts of aggregated γ-globulins up to the area of maximal precipitation. It then, paradoxically, decreased, despite the addition of more 'anti-complementary' material, thus mirroring the decrease of precipitate. When the precipitating reaction was carried out completely at 4° C., the anti-complementary effect was much decreased (Fig. 1) and failed to mirror the amount of precipitate, which remained unchanged. This suggested a marked

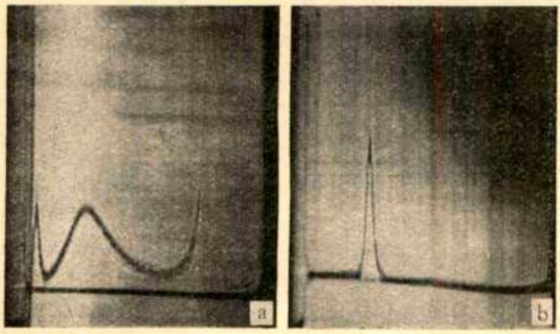

Fig. 2. Ultracentrifugal patterns of: (*a*) redissolved precipitate showing rapidly sedimenting aggregates and a small 10·5 *S* peak. Photograph taken during acceleration to 52,640 r.p.m. (*b*) purified 10·5-*S* precipitating component after removal of aggregates. Photograph taken after 32 min. at 52,640 r.p.m.

dependence on temperature for at least one step in the 'anti-complementary' effect of aggregated γ-globulins.

Fractional inactivation of components of complement[7] revealed that heat-inactivated serum (56° C. for 20 min.) deficient in the first and second components of complement, and the supernatant of serum dialysed against 0·02 molar phosphate buffer, *p*H 5·4 ('end-piece'), deficient in the first component of complement, failed to form a precipitate with aggregated γ-globulins. The redissolved precipitate ('mid-piece'), deficient in the second component of complement, formed a precipitate with aggregated γ-globulins, although 20–40 per cent less than untreated serum, and zymosan-inactivated serum, deficient in the third component of complement, and hydrazine-inactivated serum, deficient in the fourth component of complement, formed a precipitate with aggregated γ-globulins, with little or no loss of activity. These results suggested the possible identity of the precipitating factor for aggregated γ-globulins and the first component of complement. Since fixation of the latter is known to require calcium ions and to be inhibited by the chelating agent ethylenediamine tetraacetic acid[8], quantitative precipitation reactions were carried out with serum containing 0·01 *M* ethylenediamine tetraacetic acid (Fig. 1). The amount of precipitate was thereby increased, while the anti-complementary effect, measured in 50 per cent units after addition of a slight excess of calcium and magnesium ions to the supernatants, was much decreased, but not completely inhibited. These findings indicate that the precipitating factor, although suggestively similar to the first component of complement, is distinct from it as well as from the other hitherto known components of complement.

The precipitates were soluble in excess aggregated γ-globulins and in glycine buffer, *p*H 3. Ultracentrifugal examination of the acid solutions (Fig. 2) revealed a major component with an *s* rate of 9·5–10·5 *S*, large amounts of rapidly sedimenting aggregates, and frequently small 7 *S* and 19 *S* peaks. Following the removal of the aggregates by centrifugation, the major component had an $S^0_{20,\ 0\cdot15\ M\ \text{sodium chloride}}$ of 10·5 *S* and could again form a precipitate with aggregated γ-globulins. Similar findings have been reported independently recently by Müller-Eberhard and Kunkel[9].

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BIOLOGY

Five Less-Common *Salmonella* Serotypes from Congo Reptiles

DURING work on dried specimens of cloacal contents collected in the Parc National de la Garamba, north-eastern Congo, from various reptiles, the following serotypes of *Salmonella* were isolated: *champaign* (39 : k : 1,5) from a snake; *Crotaphopeltis hotamboeia*; *plymouth* (46 : d : 1,6) from the same specimen of snake and from a lizard, *Mabuya* sp.; *ramat-gan* (30 : k : 1,5) from the same snake specimen; *tel-aviv* (28 : y : enz_{15}) from a different specimen of the same species of snake; and *gatow* (6,7 : y : 1,7) from a snake *Psammophis subtaeniatus sudanensis*. The identifications were made by the Enteric Laboratory, State of Illinois, Department of Health, and confirmed by the Enteric Laboratory, Communicable Disease Center, Chamblee, Georgia.

This is the second known report of the occurrence of serotype *ramat-gan*, the first isolations having been from snakes in Israel[1]. Likewise this is the second known occurrence of serotype *gatow*, which was first isolated from the sewage of Berlin-Gatow by Hofmann and is not yet formally published although confirmed by Kauffmann[2]. The *champaign* serotype, first described from chicks by Edwards in 1945, has been reported no more than ten times, from fowl, man, diarrhœa of infants, and a bird in a zoo, and from such widely separated places as the United States, Australia and Ceylon. Serotype *plymouth* has so far been published only in a list of new serotypes issued by Kauffmann[3]. There are only a half-dozen references to serotype *tel-aviv*, three of these being from Israel, where it was twice found in chicks and later once in man. It has been reported from West Germany, France and the United States. In addition to cultures from man and chick, this type has been isolated from dog fæces, bone meal and diarrhœa of children.

The specimens of cloacal contents were collected by soaking sterile disks of filter paper (Schleicher and Schüll No. 740-*E*) and enclosing each disk in a 'Cellophane' coin envelope. On arrival by air mail in Chicago, the disks were transferred to 10-ml. brain heart infusion, incubated at 30° C., and plated at intervals on deoxycholate agar in plates of 150 mm. diameter. From each plate about 30 well-isolated colonies were picked at random for identification. Of 587 strains classified biochemically, 267 were *Salmonella*.

In general, the biochemical reactions obtained were those typical of *Salmonella*. The few exceptions are noteworthy. All 15 cultures of serotype *champaign* were indole-positive although the other reactions were typical. All but a few of 83 cultures of *gatow* gave a weak but unmistakably positive indole test in 1 per cent tryptone broth cultures 48 hr. old, using the Kovacs test. A few *gatow* cultures were negative to lysis by *Salmonella* O-phage I[4]. A few of the many *plymouth* colonies tested gave positive tests for action on gelatin, and a few others were negative with O-phage I. Some *tel-aviv* cultures were also not lysed by that phage, but all the *champaign* cultures were sensitive, as were the cultures of *ramat-gan*. Several cultures of *tel-aviv* were salicin-positive and one was positive in both salicin and sucrose. During serotyping, it was observed that a number of strains of *plymouth*, *gatow* and *tel-aviv* were monophasic and required growth in homologous phase serum broth before the alternate phase could be demonstrated.

Most of the *Salmonella* types were represented by considerable collections. Picking 30 colonies from each plate produced duplicate isolations that would not occur by the usual method of picking single representative colonies of every kind seen. Further replication occurred because the same broth culture was plated several times after different incubation periods. With the single exception of the indole reaction of *champaign*, the majority of the strains of any serotype showed typical *Salmonella* characteristics. If only a single colony had been picked, then only the atypical reactions might have been observed and a conclusion drawn that reptilian *Salmonella* exhibited atypical characteristics. In describing new serotypes on the basis of single colonies, errors may thus creep into the description. It would seem advisable to follow the zoologists, particularly the entomologists, who emphasize the need to describe new species on the basis of suitable collections rather than single specimens, so that variations can be assessed.

The extent of distribution of *Salmonella* among reptiles is remarkable. In the present work *Salmonella* were recovered from two of three snakes of one species of *Crotaphopeltis*, one of *Psammophis*, and from a lizard, *Mabuya*. They were not recovered from single specimens of *Natriciteres* and *Philothamnus*. *Salmonella* have been isolated from snakes of the latter genus by LeMinor, Ravisse and Drean[5], who also found *Salmonella* in *Crotaphopeltis*. In their series of 575 snakes from Ethiopia, Eritrea and Lybia, Serie and LeMinor[6] found no *Salmonella* in snakes of the genus *Nahia*, but did find them in various snakes of the families Colubridae and Viperidae.

The investigation of which the present observations are a part is being made to explore the question: What is the bacterial flora of reptiles, and what is the significance of the presence of *Salmonella*? The extensive literature already shows that reptiles harbour *Salmonella*. Are these part of the natural intestinal flora of reptiles, or are they acquired through association with rodents, man or other sources? The herpetologist who collected the specimens and identified the reptiles felt certain that the chance of infection from man was negligible in the area of the Parc National de la Garamba, and that the specimens came from animals which would be extremely unlikely to feed on rodents. The present work is therefore believed to suggest that *Salmonella* are naturally present in the reptilian intestine, possibly as essentially harmless commensals.

For the opportunity to examine this material, grateful acknowledgment is made to the authorities of the Parc National de la Garamba (director, Dr. V. Van Straelen), Brussels, Belgium. The specimens were collected by Dr. R. F. Inger, curator of amphibians and reptiles, Chicago Natural History Museum, who also identified the reptiles. The work was supported in part by a grant (*E*-2437) from the National Institutes of Health, U.S.A.

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Use of Polyethylene Glycol in Biology

IN a recent paper, I. Mackenzie Lamb[1] directed attention to the use of polyethylene glycol ('Carbowax') for the permanent preservation of algal material for microscopic study. He shows that it is practicable to preserve algae in the dry state embedded in polyethylene glycol and mounted on cards. When a portion is required for study, part of the card is snipped off and soaked in water. The alga then resumes its original state sufficiently well for detailed microscopic examination.

It seemed that such a technique could be expanded with advantage into a much wider field, and accordingly some simple experiments were carried out on plankton preserved in formalin in Aberdeen. Mixed phytoplankton, and individual copepods, decapod larvæ, chætognaths, medusæ and ctenophores were used. Phytoplankton from a preserved sample was added to a drop of 30 per cent polyethylene glycol on a slide and put aside to dry. The individual zooplankton organisms were similarly treated on slides, in a watch glass or evaporating dish according to size. Some were flooded with water the next day, others left for periods varying from a week to three months. Recovery takes only seconds on the slides, and a few minutes for the larger forms.

The phytoplankton samples were all in excellent condition and there was no difficulty at all in identifying the various species, including the delicate invertebrate larvæ, etc. The texture as seen with an oil immersion lens was surprisingly good. The chætognaths also recovered extremely well; the fin-rays, cellular structure of the corona and other features could be determined just as easily as in the originals, and there was no difficulty at all in dissecting out the teeth. Copepods and decapod larvæ also recovered well, perhaps somewhat shrivelled but not seriously so, and certainly well enough for easy dissection of the appendages. Medusæ and ctenophores did not recover their original size and shape and remained very shrivelled, but the texture of the tentacles of the medusæ and the combs of the ctenophores remained good and identification would be possible.

Such experiments are extremely crude and should be repeated in greater detail and with a wider range of species, but the method has such attractive possibilities that it is worth bringing it now to the notice of as many workers as possible.

A 30 per cent solution of polyethylene glycol is an excellent medium in which to examine and dissect specimens under a microscope. It penetrates quickly and clears the organism to what, in my opinion, is the ideal state for handling, leaving the fine setæ still visible. It is easy to lift the dissected parts through the surface film with a needle for further examination.

In addition to Lamb's technique for the dry storage of algae on cards, the following uses of polyethylene glycol are envisaged.

(1) Specimens of those types that recover well can be sent dry by post without special packing, and with a much-reduced risk of loss due to breakage of the jar or other container, and no risk of damage by leaking formalin to other goods in the post. Delicate or intricate specimens can be sent in a block of polyethylene glycol with no risk of displacement of parts.

(2) Micro-specimens can be sent dry, on a slide, to be recovered at the destination in such a form that they can be handled or examined by any desired technique.

(3) Parts of specimens dissected in a 30 per cent solution cannot come to harm by drying in transfer to the slide from a needle point, nor will they, or the specimen, be harmed if the observer has to leave his work to attend to something unexpected and of immediate priority. If this should result in his being unable to return to the work until after it has dried up, there is then no difficulty in resuming.

(4) Even carefully sealed museum tubes are liable to dry if not examined at regular intervals, and a minute crack, unseen at the time, can result in the complete loss of the specimen for most purposes. If preserved with polyethylene glycol added, there is a good chance that the loss would not be so serious, and a little more polyethylene glycol than the equivalent volume of the specimen simply added to the preservative should be a worth-while insurance.

It is also known as 'Carbowax' and is available in various melting points; Lamb suggests that that known as 'Carbowax 4000', melting point 55° C., is the most suitable. It is a white, rather waxy substance which dissolves readily in water, formalin and 70 per cent alcohol (but not in absolute alcohol). Specimens can be transferred to a solution at room temperature and allowed to dry if desired. For embedding in blocks, they can be transferred direct to the melted polyethylene glycol in a bath at 55° C. In Britain the cost is about 16*s.* per kgm., so that it is not expensive for so versatile and useful a commodity.

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β-Glycosidase Activity and Cyanogenesis in the Susceptibility of Alfalfa to Winter Crown Rot

Hydrogen cyanide was demonstrated by Lebeau and Dickson[1,2] in alfalfa crown buds infected with an unidentified basidiomycete. They showed that a close association existed between the presence of hydrogen cyanide and mycelium in the infected buds, but did not determine whether the cyanide was produced by the fungus or host or was due to an interaction between the two. The experiments reported here were designed to clarify this problem.

Preliminary experiments were carried out to determine β-glycosidase activity of a crude enzyme preparation of the fungus cultured on a synthetic medium. Glycosidase activity of a crude enzyme preparation, obtained as the supernatant from centrifuged (13,000*g*) seven-day-old shake cultures, was determined by a modification of the Conway diffusion method[3]. 2 ml. of the crude enzyme and 1 ml. of a 0·02 *M* amygdalin solution were placed in the outer chamber and 1 ml. of alkaline sodium picrate[4] in the centre well of a Conway unit. Release of cyanide was indicated by a red coloration of the sodium picrate, which reached a maximum in 30 min. Activity of the crude enzyme preparation was not affected by dialysing but was destroyed by boiling.

Hydrogen cyanide has been reported from species of *Medicago*[5], and therefore a series of experiments was carried out to determine the effect of the crude enzyme on filtrates of *Medicago sativa* and *M. falcata*. Alfalfa crown buds obtained from plants under snow cover were homogenized for 30 min. in a Waring blender with sufficient distilled water added to facilitate homogenation. The homogenate was filtered through a double layer of cheese-cloth and the filtrate divided into two equal aliquots. Each aliquot was made up to 200 ml. with distilled water and transferred to a 500-ml. suction flask. Approximately 100 ml. of crude enzyme was added to the filtrate in one flask and an equal volume of distilled water to the other. Air was drawn through the reaction mixtures, passed through absorbent cotton and bubbled through 20 ml. of alkaline sodium picrate to indicate hydrogen cyanide production. Aeration was carried out at 15° C. for 48 hr. Control flasks containing distilled water or the crude enzyme were similarly treated. The optical densities of the picrate solutions were determined at 480 mμ using a Bausch and Lomb colorimeter. The results are shown in Fig. 1.

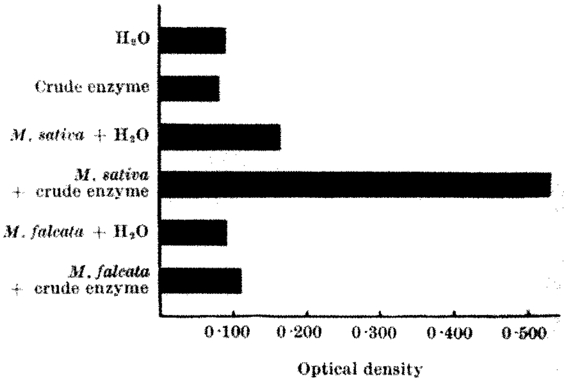

Fig. 1. Hydrogen cyanide production from filtrates of tissue homogenates of *Medicago sativa* and *M. falcata*

Hydrogen cyanide was released in an appreciable amount only from the *M. sativa* filtrate. Lack of activity with the *M. falcata* filtrate suggests that this material may have a lower glycoside content or, alternatively, contains a β-glycosidase inhibitor or a detoxification mechanism for cyanide. It appears likely therefore that production of hydrogen cyanide in infected alfalfa plants in the field is due to β-glycosidase activity on the part of the fungus and the provision of cyanogenic substrates by the host. The difference in response of the two filtrates is of particular interest since *M. falcata* is comparatively resistant to winter crown rot in the field.

Further work is being carried out, and detailed results will be reported in due course.

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Ethylene Production by Cytoplasmic Particles from Apple and Tomato Fruits in the Presence of Thiomalic and Thioglycolic Acid

Despite several tracer studies[1,2] and the use of very sensitive techniques for the detection of ethylene[3,4], there has been no clear indication of a biosynthetic pathway that can evolve ethylene in fruits. Spencer[5] reported ethylene production by cytoplasmic particles from tomato fruit in the presence of malate and co-factors. This suggested that the biosynthesis of ethylene could be related to the metabolism of organic acids in the cells. Tracer experiments by Burg[2], however, showed no evidence linking ethylene evolution to Krebs cycle metabolism. Meigh *et al.*[4] could not detect ethylene in incubation mixtures containing apple mitochondria, malate and co-factors. The latter experiments are especially significant since they used apple mitochondria known to metabolize Krebs-cycle substrates, and the system for detecting ethylene was considerably more sensitive than that used in any of the other investigations. It therefore appears that ethylene synthesis in fruits must be associated with some other aspect of organic acid metabolism.

Recently we have isolated cytoplasmic particles, from apples and tomato fruits, that evolve ethylene in the presence of thiomalic and thioglycolic acid. Cysteine, glutathione, methionine, and other related compounds (thiodiglycolic acid, α-monothioglycerol, β-mercaptopropionic acid, carboxymethylmercaptosuccinic acid) did not replace the requirement for thiomalic or thioglycolic acid. Results of a number of experiments with particles from apples are summarized in Table 1. Similar results were obtained with particles from tomato fruit.

Table 1. Ethylene Production by Cytoplasmic Particles from Apples in the Presence of Thiomalic and Thioglycolic Acid

Flask content*			Ethylene† (µl./mgm. N/hr.)
Thiomalic	+ co-factors	+ no particles	0·0
Thioglycolic	+ ,,	+ no particles	0·0
Thiomalic	+ ,,	+ particles	0·019
Thioglycolic	+ ,,	+ particles	0·016
Thiomalic	+ ,,	+ denatured particles	0·0004
Thiomalic	+ ,,	− PO_4 + particles	0·004
Thiomalic	+ ,,	+ DIECA + particles	0·003
Thiomalic	+ ,,	+ DIECA + 10^{-5} *M* copper sulphate + particles	0·006

* The flask contents consisted of the following: 50 µm. thiomalic or thioglycolic acid, 3 µm. adenosine triphosphate, 60 µm. phosphate, 0·08 µm. coenzyme-*A*, and 400 µm. sucrose. 1 ml. of particulate suspension containing approximately 1·5 mgm. nitrogen was added to each flask. The flasks were incubated 2 hr. at 30° C. in an automatic shaker. The *p*H of the reaction mixture was 6·2.

† Ethylene was determined by gas chromatography with a system previously described (ref. 4). The method was sensitive to 3 × 10^{-6} µl. of ethylene in a 1-ml. sample.

These ethylene-producing systems have many characteristics of an enzymatic process. The rate of ethylene production is proportional to the concentration of the particles. The rate, during a 45-min. period, is approximately proportional to the length of the incubation, and the reaction is essentially inhibited by heat denaturation. The reaction appears to be stimulated considerably by phosphate. Furthermore, some chelating agents that inhibit enzymatic reactions, such as diethyldithiocarbamate (DIECA) and ethylenediamine tetraacetic acid (EDTA), markedly inhibited this reaction and the inhibition is partially reversed by the addition of copper to the system.

It is noteworthy that these preparations did not oxidize Krebs cycle substrates. Another important difference between mitochondria and these particles is in the *p*H of the isolating media. Whereas active mitochondrial particles are isolated from homogenates of neutral or slightly alkaline media, it was found necessary to isolate these particles from homogenates of acid media. Particles that cause the evolution of ethylene were obtained from fractions precipitated at 500*g*, 16,000*g* and 40,000*g*.

These findings offer a completely new approach to the study of ethylene biosynthesis. Work is now in progress on the purification of the enzyme or enzymes and the elucidation of the mechanism of the reaction by tracer techniques.

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ENTOMOLOGY

Ribonucleic Acids in the Moult Cycle of an Insect

It is well known that the cuticle in insects is shed at the end of each instar and a new one is formed by the underlying epithelium. Although much is known about the histological changes in the epithelium and the chemical changes in the cuticle during this complicated process of moulting[1,2], nothing is known about the role of the ribonucleic acids (RNA) during this period. Since it is known that RNA plays an important part in cellular protein synthesis[3], it was considered desirable to study the RNA of the insects during the moult cycle.

In the present investigation the nymphs of *Gryllus bimaculatus* were used. Young nymphs, hatched within a period of 15 hr., were separated and maintained in the laboratory under identical conditions of temperature, light, humidity, etc., so that their rate of growth would be the same. Prior to the extraction of RNA, the nymphs were homogenized and treated successively with 2 per cent perchloric acid and an alcohol–ether mixture in order to remove the nucleotides and the lipids respectively. RNA was extracted by treating the residue, in the cold, with N perchloric acid for 18–24 hr. and estimated by the ultra-violet absorption and/or the orcinol method in the presence of ferric chloride. For purposes of comparison, total nucleic acids were extracted with warm 0·5 N perchloric acid and RNA was estimated by the orcinol reaction[4]. Histological preparations of nymphs fixed in Zenker's fluid have also been made and were stained with Unna's methyl green–pyronin stain.

The first-stage nymphs of *Gryllus bimaculatus* moult approximately at the end of the fifth day after hatching, while those of the second stage moult, usually, on the sixth or seventh day after the first moult. There is considerable individual variation in the time taken by the nymphs in the succeeding stages. Hence, for the quantitative estimations of the RNA, only individuals of the first two stages were used during this work.

Results are given in Table 1.

Table 1

Age of the nymph after hatching or moulting (days)	RNA content per mgm. weight of nymph (μgm.)	Total RNA per individual (μgm.)
1	7·2	5·6
2	5·1	4·6
3	4·5	8·1
4	0·7	1·3
5	0·7	1·3
Soon after first moult	0·8	1·6
1	2·1	6·3
2	2·4	7·5
3	5·4	21·2
4	7·6	32·0
5	2·4	11·0
6	0·7	3·3
Second moult		

It can be seen from Table 1 that the RNA content per mgm. wet weight of the nymph gradually falls from the time of hatching to the first moult, and then rises to a peak at the intermoult period; it decreases once again to a minimum at the time of the second moult. However, if we consider the total ribonucleic acid content per individual, it is evident that the RNA content reaches its highest figure, both in the first- as well as in the second-moult stage nymphs, at the middle intermoult period, that is, before the nymph is actually preparing for the succeeding moult. There is thus a gradual accumulation of RNA until the nymphs are preparing for the moult, and a sudden fall during the pre-moult period.

The histological preparations stained with Unna, after treatment with ribonuclease, or with buffer or without any pretreatment, showed a similar fluctuation in the ribonucleic acid content as indicated by the intensity of staining. The nucleoli in the chitogenous epithelium, which are prominent by their strong pyroninophily in the intermoult period (Fig. 1) are less visible in nymphs preparing to moult and in those which have just emerged from the moult (Fig. 2). Staining of the newly moulted (early post-moult stage) nymphs of the first five stages also showed faint staining of the nucleoli when compared with those of the intermoult stage nymphs. As the range of individual variation in the time taken for moult from the third stage onwards is great, day-to-day observations were no longer useful. However, such observations of the first two stages suggest that the nucleoli gradually become more prominent after the moult until the onset of the succeeding moult. On the contrary, the cytoplasm of the epithelial cells shows greater avidity for pyronine when the new cuticle is being laid, that is, just before and soon after the moult; while in the intermoult period, it is less pyroninophilic. In the adult insects of varying ages the epithelium shows only faint staining with pyronine both in the nucleoli and the cytoplasm.

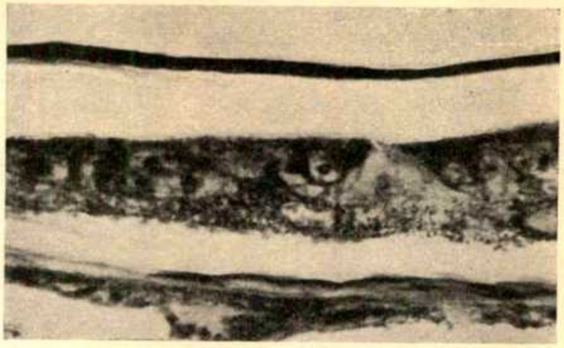
Fig. 1. Chitogenous epithelium of an intermoult second-stage nymph stained with Unna

The results reported above suggest that there is a gradual accumulation of RNA in the nymph from the time of moult until the late-intermoult period, and then a sudden decrease. Observations with methyl green–pyronin further show that the basophilia of the nucleolus and of the cytoplasm undergoes cyclic changes during the moult cycle, suggesting that nucleolar RNA might be transferred to the cytoplasm at the pre-moult stage. Finally, these observations indicate that the hormone which is secreted by the prothoracic glands and which is known to reach a titre sufficient to cause moulting some time before the processes involved in moulting are actually initiated[5,6] might influence the release of nucleolar RNA into the cytoplasm.

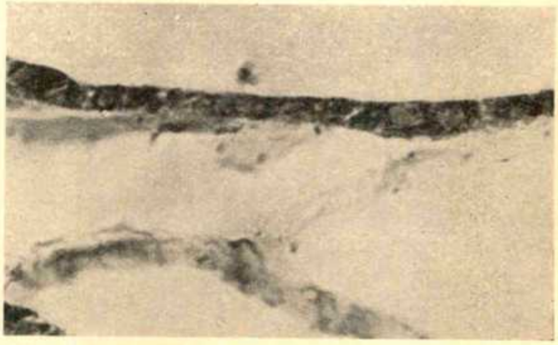
Fig. 2. Chitogenous epithelium of an early post-moult second-stage nymph stained with Unna

I thank Prof. J. Brachet for guidance, encouragement and facilities.

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Trans-2-Hexenal in the Scent Gland of the Hemipteran *Acanthocephala femorata*

The plant bug, *Acanthocephala femorata* (Fab.), is one of the many hemipterous insects which eject odoriferous materials when disturbed. This large brown coreid, which occurs in the southern United States, is frequently found on citrus plants[1].

The scent gland secretion of *Acanthocephala* adults is stored in a large round reddish-orange sac which lies on the floor of the body cavity covering the metathoracic and first abdominal segments and extending to the second abdominal segment. This sac opens to the outside of the body-cavity through a pair of ostioles each of which is located on a suture between the sternal plate and the pleural plate of the metathorax. The ducts leading from the sac to the ostioles enlarge just inside each ostiole and can be opened to the outside by means of muscle-operated lips. The sac itself contains no obvious musculature for compression which would be required for ejection of the secretion. However, it was observed that ejection always occurred when the metathoracic legs were flexed. If the metathoracic legs were held immobile, no ejection occurred. Indeed, it was observed that movement of metathoracic coxæ from which the femora had been severed was coincident with ejection of the secretion. It is possible that the force required for ejection of the secretion is supplied by coxal pressure during contraction of the metathoracic leg; the secretion thus would be ejected when the lips of the sac ducts are simultaneously opened.

The secretion of *Acanthocephala* adults and nymphs issues as fine droplets. The adult secretion can be sprayed for a distance of at least 8 in. If one side of the body is mechanically stimulated, the spray is unilateral from the stimulated side. However, if the dorsum of *Acanthocephala* is stimulated, the spray is often bilateral.

Samples of clear yellow fluid from the sac were obtained by removing the abdominal dorsum and viscera of *Acanthocephala* and piercing the exposed sac with a fine capillary. Fluid collected in capillaries was identified as *trans*-2-hexenal by infra-red spectrophotometry, mass spectroscopy and the 2,4-dinitrophenylhydrazone derivative.

For infra-red spectrophotometry, a film of the secretion was used. The resulting spectrum demonstrated that the *Acanthocephala* secretion was a conjugated aliphatic aldehyde exhibiting *trans* deformation. The absorption bands were: aldehyde C—H, 3·66μ; C=O, 5·89μ; C=C, 6·08μ; *trans* C=C, 10·25μ. The spectrum was identical to that of 2-hexenal[2].

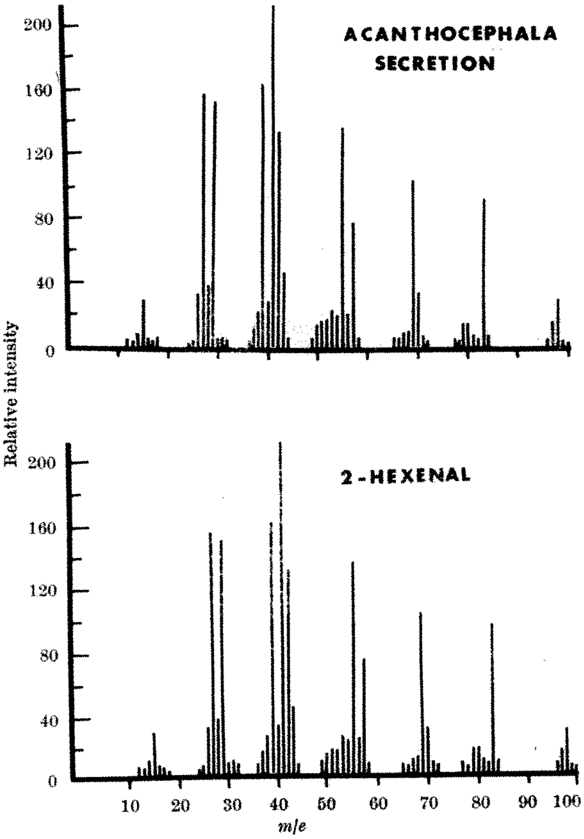

Fig. 1. Comparison of the mass spectra of 2-hexenal and the *Acanthocephala* secretion

The compound was analysed in a modified Consolidated 21–102 analytic mass spectrometer by injecting the compound with a microsyringe into the heated inlet system (100° C.) of the instrument. A normal magnet run at a pressure of 65·22μ indicated no masses above 98. A high magnet run further established the absence of any higher boiling components. Analysis of the structural fragmentation pattern established the *Acanthocephala* secretion as a hexenal which was identical with that of synthetic 2-hexenal (Fig. 1). Virtually superimposable fragmentation patterns indicated that the *Acanthocephala* secretion was a pure enal.

The 2,4-dinitrophenylhydrazone of the enal was prepared by adding an alcohol solution of the secretion to a saturated solution of the reagent in 2 *N* hydrochloric acid. The purified derivative melted at 144–145° C. uncorrected and did not produce a melting point depression in admixture with the derivative of synthetic 2-hexenal melting at 145–146° C.[3]. The elementary analysis of the *Acanthocephala* derivative corresponded to hexenal (found: C, 51·52; H, 5·15; N, 19·89; calculated for $C_{12}H_{14}N_4O_4$, C, 51·79; H, 5·04; N, 20·14 per cent).

2-Hexenal has also been found in the cockroach *Eurycotis floridana*[2]. In this insect the secretion is stored in a sac which opens to the outside of the body between the sixth and seventh abdominal sternites. It is of interest to note that males of the giant water bug *Lethocerus indicus* (Lepeletier and Serville) produce the acetate ester of 2-hexenal, which is given off through tubules located in the abdomen[4]. Elsewhere, 2-hexenal has been found in whale oil[5] and is a common constituent of plants[3].

Nothing is known of the function of 2-hexenal in Nature. It may serve as a repellent for predators of *Acanthocephala*. In the laboratory, *Acanthocephala* sometimes ejected this compound at imported fire-ants (*Solenopsis saevissima* var. *richteri* Forel) which were approaching. The ejection was unilateral to the side facing the ants and caused the ants to move away. When the ants actually attacked *Acanthocephala* it always ejected copious quantities of the secretion.

We are indebted to Dr. R. P. Curry of the Ethyl Corporation, Baton Rouge, Louisiana, for helpful discussion of the infra-red spectra and Prof. P. S. Callahan of Louisiana State University for morphological interpretations.

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BACTERIOLOGY

Hæmolytic Activity of *Corynebacterium ovis*

THE Priesz–Nocard bacillus (*C. ovis*) which is responsible for a number of diseases of animals, including caseous lymphadenitis of sheep and ulcerative lymphangitis of horses, grows well on blood-agar medium, producing circular, opaque surface colonies which are frequently surrounded by a narrow zone of hæmolysis.

The hæmolysin, which is active against guinea pig, rabbit, horse and sheep corpuscles, is thermostable, non-antigenic and appears to be closely linked to the bacterial cells so that it cannot be obtained free in a filtrate[1].

During an investigation of the effects on animal erythrocytes of combinations of diffusible substances produced by bacteria (to be published), it was observed that mixed cultures of *C. ovis* and *C. equi* showed a synergistic hæmolytic effect on sheep, goat, ox and rabbit cells. The phenomenon was better illustrated by streaking the strains at right angles to each other on the surface of 1·5 per cent nutrient agar plates containing 4 per cent red cells which were previously washed three times in normal saline and re-suspended to the original volume of the blood. The inoculated plates were incubated in air-tight jars in a mixture of 10 per cent carbon dioxide and 90 per cent air, at 37° C. for 24–48 hr.

Fig. 1 shows the effect on sheep blood agar after 48-hr. incubation with the vertical culture streak of *C. ovis* running through the horizontal streak of *C. equi*. The hæmolytic pattern obtained is the result of the interaction of the diffusible products of both strains; that of *C. ovis* being active over a larger area than *C. equi* and neither exerting the hæmolytic effect independently. Unlike the hæmolysin described by Carne, the diffusible substance produced by *C. ovis* is active against ruminant and rabbit cells, but not horse red cells, and its effect is enhanced by the presence of 10 per cent carbon dioxide.

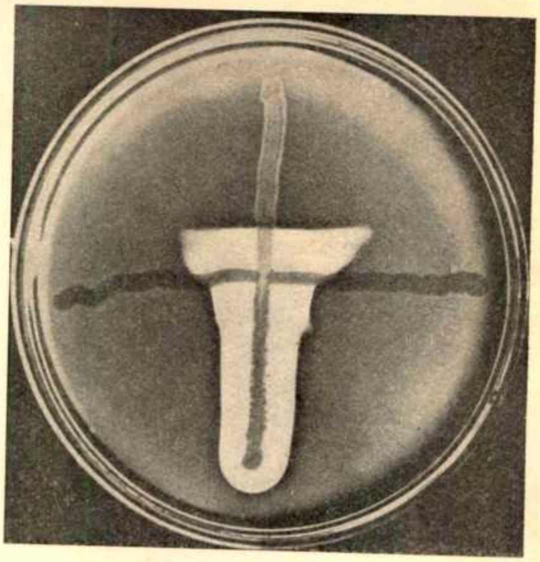

Fig. 1. Sheep blood-agar plate showing the hæmolytic effect of *C. ovis* in the presence of *C. equi*

The plate method, which appears to be more sensitive than tube titration, has also been used to show that the diffusible product of *C. ovis* is filtrable and is capable of dialysation through 'Cellophane'; and is being adapted for antigenic studies.

These preliminary observations suggest that an indicator system of animal erythrocytes previously treated with an actively growing culture of *C. equi* may prove of value in elucidating the true nature of the hæmolysins of *C. ovis*.

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Inhibition of Multiplication of Bacteriophage by Actinomycin

IT has recently been found that actinomycin forms a complex with deoxyribonucleic acid but not with ribonucleic acid[1]. In this communication we wish to present evidence to show that the antibiotic prevents multiplication of the phage while permitting the growth of the host bacterium.

The actinomycin used in these experiments was crystalline actinomycin *S*[2,3], supplied by the Daiichi Seiyaku Pharmaceutical Co., Ltd. The standard methods used for handling bacteria and phage and for determining nucleic acid were those described in another communication[4].

Actinomycin *S* is known to prevent the growth of Gram-positive bacteria, but *Escherichia coli B*, a Gram-negative bacterium, used in this experiment, can grow actively in the presence of the antibiotic. Exposure of free *T2r* phage to 100 μgm./ml. of actinomycin *S* for 60 min. at 37° C. did not cause any activation of the phage. The effect of the antiiotic on phage reproduction was studied. Cells of . coli B, harvested during the logarithmic phase of rowth, were infected with $T2r$ phage and shaken at 7° C. in a glucose–salt synthetic medium with arious concentrations of actinomycin S.

The results are illustrated in Table 1, which shows hat the reproduction of $T2r$ phage is progressively npaired by increasing concentrations of actinomycin , while the synthesis of deoxyribonucleic acid in the hage-infected cells is not affected by the antibiotic. t must be emphasized that the infected bacteria roduce deoxyribonucleic acid in a similar way even when the antibiotic is added 5 min. either before or after infection.

able 1. EFFECT OF ACTINOMYCIN S ON THE MULTIPLICATION OF HAGE AND THE SYNTHESIS OF DEOXYRIBONUCLEIC ACID BY $T2r$-INFECTED BACTERIA

Concentration of actinomycin S (μgm./ml.)	Relative increase (percentage of control)	
	Phage titre	Amount of deoxyribonucleic acid
0	100	100
1	45	—
3	29	—
10	12	98
30	10	94
100	4	87

The lysate obtained in the presence of a level of 30 μgm./ml. of actinomycin S contained nearly the same amount of the deoxyribonucleic acid as that found in the untreated control lysate, but the infectivity of the former lysate was only one-tenth of the latter. When the lysates were centrifuged at 3,000g for 15 min., the majority of the deoxyribonucleic acid was found in the supernatant fluid, both in the presence and in the absence of the antibiotic. Hence analyses were made on these supernatant fluids. As shown in Table 2, a significant difference between the control and the antibiotic-treated lysates was revealed by comparing the amount of deoxyribonucleic acid in the sediment obtained by centrifugation at 20,000g. At this gravity, normal $T2r$ phage but not free nucleic acid was sedimentable. Furthermore, the deoxyribonucleic acid which had been produced by infected cells exposed to the antibiotic was not precipitable by anti-$T2r$ phage serum and was sensitive to deoxyribonuclease. These results indicate that this deoxyribonucleic acid has no protein coat reacting with the antiserum and protecting the viral deoxyribonucleic acid from deoxyribonuclease.

These effects of actinomycin S on phage reproduction are essentially similar to those of proflavine[5]. One may suppose that actinomycin S, like proflavine, blocks some final step in the maturation of the phage.

Table 2. PROPERTIES OF DEOXYRIBONUCLEIC ACID IN THE LYSATE MADE IN PRESENCE OF ACTINOMYCIN S

Fraction	Deoxyribonucleic acid found*	
	Control lysate	Lysate with actinomycin S
Initial lysate	100	100
Low-speed centrifugation supernatant	90	73
Sediment at 20,000g	34	2
Precipitate with antiserum	53	2
Deoxyribonuclease-resistant	39	10

* Values in each fraction represent percentage of the amount of deoxyribonucleic acid in the initial lysate.

However, there is a significant difference between the action of two inhibitors; the formation of infective phage is reversed by removal of proflavine, but not by removal of actinomycin S. Thus further work is necessary to determine whether normal viral protein is produced by the antibiotic-treated cells.

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VIROLOGY

Effect of an Organic Gold Salt on Eaton's Primary Atypical Pneumonia Agent and Other Observations

Eaton, Liu and their colleagues have provided evidence that the causative agent of a certain type of human primary atypical pneumonia, namely, that in which a proportion of patients develop serum agglutinins against *Streptococcus MG* and human erythrocytes at 4° C., is filterable, can be propagated in the lung of the chick embryo, and produces lung lesions when inoculated into hamsters and cotton rats by the intranasal route[1]. Some uncertainty exists about the nature of this organism. The strains studied had a large particle size (between 180 and 250 mμ as measured by filtration[1b]); some were sensitive to streptomycin[2] and all to tetracyclines[3].

We have observed that the macroscopic and microscopic lesions produced in the hamster lung by the intranasal inoculation of chick embryo lung infected with the 'Hetter' strain of Eaton's primary atypical pneumonia agent resemble in some respects those seen in the lungs of mice infected intranasally with cultures of pleuropneumonia-like organisms[4].

The size of Eaton's organism is of a similar order to that given for the elementary particles of some pleuropneumonia strains (125–250 mμ), and its pattern of antibiotic sensitivity is also similar to that of the pleuropneumonia group[5]. Eaton and his colleagues were unable, however, to grow pleuropneumonia-like organisms from egg material containing strains of the organism[1].

It has been shown that organic gold salts will suppress lung[6] and other lesions[7] produced by pleuropneumonia-like organisms in animals, and accordingly we have investigated the effect of sodium aurothiomalate ('Myocrysin', May and Baker) on experimental infections with Eaton's primary atypical pneumonia agent in hamsters and chick embryos. The 'Hetter' strain of Eaton's agent, obtained from Dr. C. Liu and kindly given to us by Prof. C. H. Stuart-Harris (Sheffield), was used for all the experiments and was passed by the amniotic route in 13-day-old chick embryos. Infection was recognized in frozen sections of the lungs of the chick embryos by using the indirect fluorescent antibody method with a 'middle layer' consisting either of a pool of convalescent-phase sera from local patients with primary atypical pneumonia or, for checking, of a specific rabbit antiserum against the classical 'Mac' strain kindly provided by Dr. M. D. Eaton (Boston).

Groups of hamsters were inoculated intranasally with 0·6 ml. of a 10 per cent suspension of infected chick embryo lung. Some groups were given a daily dose of 3–5 mgm. of sodium aurothiomalate intraperitoneally. Others were kept as untreated controls or injected with the excipient without the gold salt. All groups were killed 11 or 12 days after intranasal inoculation and the intensity of their lung lesions graded on two arbitrary scales depending on (*a*) the macroscopic degree of lung consolidation, and (*b*) the proportion of bronchi and vessels with cuffs of mononuclear cells and the degree of alveolar consolidation seen in lung sections.

Table 1 shows the results obtained in experiments with four different egg passage-levels of the 'Hetter' strain. There was a consistent reduction in the intensity of the lung lesions in hamsters treated with gold. The lung lesions were also reduced to a similar degree (an average macroscopic lesion score of 0·12 was obtained as part of exp. 4) by a convalescent-phase serum from a local patient (Ev. S) with primary atypical pneumonia. This serum had a fluorescent antibody staining titre of 1 in 50 to 1 in 100 when titrated on infected chick embryo lung sections ; the patient's acute-phase serum did not stain at a dilution of 1 in 5. This neutralization provides an indirect check that the lesions produced in hamsters, and inhibited by the gold salt, were in fact caused by the 'Hetter' organism and not by a second (hypothetical) organism present in the egg material but unrelated to Eaton's agent.

Table 1. EFFECT OF SODIUM AUROTHIOMALATE ON THE LUNG LESIONS PRODUCED IN HAMSTERS BY EATON'S PRIMARY ATYPICAL PNEUMONIA AGENT

Exp. No.	Controls			Gold-treated		
	No. of animals	Average lesion score		No. of animals	Average lesion score	
		Macroscopic	Microscopic		Macroscopic	Microscopic
1	3	1·5	4·6	4	0·25	1·5
2	8	1·6	6·0	7	0·14	1·0
3	9	1·7	5·8	9	0·3	1·2
4	9	1·6	4·9	7	0·07	1·1

Analysis of the frequency distribution of the individual lesion scores for all fifty-six hamsters indicates that the differences between treated and untreated groups were unlikely to have arisen by chance alone. Macroscopic lesion scores : $\chi^2 = 31·5$; d.f. $= 5$; $P < 0·001$; microscopic lesion scores : $\chi^2 = 53·3$; d.f. $= 8$; $P < 0·001$.

In other experiments a single dose of 25 or 30 mgm. of sodium aurothiomalate was placed in the yolk sac of chick embryos either before, or at the same time as, the inoculation of a suitable dilution of the 'Hetter' seed into the amniotic cavity. Subsequent examination of frozen sections of the chick embryo lungs showed that substantially fewer of the treated groups had specific antigen ('fluor-stained antigen') when examined by the indirect fluorescent antibody method. Also pools of lung tissue from the treated embryos did not produce pneumonia in hamsters, whereas those from the control, untreated embryos did so.

These results are in accord with the view that Eaton's primary atypical pneumonia agent may be biologically related to the pleuropneumonia group. However, more information on the effect of gold on accepted viruses is required, although Bauer[8] found that neurovaccinia was not inhibited.

The results of intensified Giemsa-staining of sections of chick-embryo lung infected with the 'Hetter' strain also suggest an affinity between the agent and pleuropneumonia-like organisms. Acetone-fixed, frozen sections (about 5μ) were treated with 1 per cent potassium permanganate[9] for 2 min., washed and stained with 1 in 25 Giemsa in phosphate-buffer *p*H 6·4 for 18 hr. They were differentiated with 0·5 per cent acetic acid, washed and then either mounted in a mixture of equal parts of glycerol and phosphate-buffer and examined at once or—with more distortion and variability—dehydrated with acetone, cleared with xylol and mounted in a polystyrene (*DPX*).

Minute pink coccobacilliform bodies were seen in association with the bronchial epithelial cells. Some were intracellular, but the majority appeared to be arranged as a compact layer on the surface of the cells, and they seemed to occupy virtually the same position as the fluor-stained antigen found in adjacent or other sections of the chick-embryo lungs. (In our experience the exact location of the fluor-stained antigen is difficult to judge with the fluorescence microscope. The antigen might be completely within the cytoplasm of the bronchial epithelial cells and just under the cell membrane or, alternatively, it might be predominantly on the surface of the cells with some material taken into the cells by phagocytosis.) Coccobacilliform bodies have not yet been seen in sections of uninfected chick-embryo lungs. A close correlation has, however, been observed between the presence of fluor-stained antigen and coccobacilliform bodies in embryos inoculated with a variety of positive or negative materials. The coccobacilliform bodies appear at about the same time—three to four days after inoculation—as the fluor-stained antigen in injected chick-embryo lung, and, like the antigen, they are suppressed or reduced in quantity in embryos treated with sodium aurothiomalate. Further experiments are being carried out to obtain evidence from other directions on the identity, or otherwise, of the coccobacilliform bodies and the specific fluor-stained antigen.

As these findings may be peculiar to our sub-line of the 'Hetter' strain, it now seems desirable that other strains of Eaton's primary atypical pneumonia agent should be examined elsewhere for coccobacilliform bodies by the method of mild fixation and intensified Giemsa staining, and that they should also be tested for sensitivity to organic gold salts *in vivo*.

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June 14.

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Hæmagglutination and Hæmagglutination-Inhibition with African Horse-Sickness Virus

Complement-fixation and mouse neutralization tests are the two techniques so far employed in serological work with African horse-sickness virus. It was thought worth while to study the hæmagglutinating properties of this agent with the view of obtaining yet another tool for epidemiological (epizootiological) studies of this dreaded horse disease.

The three strains studied were an Indian strain recently isolated from the lung of a dead horse, a Pakistan strain and an attenuated type 6 strain from South Africa. The latter two were kindly supplied by Dr. Alexander of the Onderstepoort Laboratory in South Africa. The Indian and the Pakistan strains had undergone approximately 20 and the type 6 strain probably 100 or more mouse brain passages.

The basic techniques for preparing antigens, sera and erythrocytes for hæmagglutination-inhibition test were those described by Clarke and Casals[1]. Antigens prepared from infected suckling mouse brains were : (1) acetone–ether extract ; (2) sucrose–acetone extract ; (3) sucrose–acetone extract treated with protamine sulphate ; (4) crude aqueous alkaline extract ; (5) the same treated with protamine sulphate. Control antigens were prepared from the brains of normal healthy mice of the same age. Erythrocytes collected from roosters, geese, sheep, guinea pigs, horse, mule and donkey were tested. The cell concentrations used ranged from 0·4 per cent to 1 per cent. The cells were suspended in 'adjusting diluents', which when mixed with an equal volume of 'serum–virus diluent' gave a *p*H range from 6·4 to 7·0. The hæmagglutination test was performed in tubes with 0·5 ml. of antigen and 0·5 ml. of required cell suspension. One set was incubated at 37° C., one at room temperature (25° C.) and one at 4° C.

Initial experiments revealed that this virus showed hæmagglutination with cells from the equines alone. As the most satisfactory pattern was obtained with horse cells, these were used for all further work. Only the two antigens (Nos. 3 and 5) treated with protamine sulphate showed hæmagglutination. Of the two, sucrose–acetone extract treated with protamine sulphate gave better results than crude extract treated with protamine sulphate. Control antigens prepared from normal mouse brains in the same manner did not show any hæmagglutination. The best results were obtained with 0·5 per cent horse red blood cells, using *p*H 6·4 and incubation at 37° C. The test was read within 1¾–2 hr., when the cell controls had settled. The three strains gave similar hæmagglutination titres ranging from 1 : 32 to 1 : 128.

Hæmagglutination-inhibition work is still in a preliminary stage. Removal of non-specific inhibitors from sera was attempted by treatment with kaolin, acetone extraction and inactivation at 56° C. for 30 min. Antigen and serial two-fold dilutions of serum were mixed in 0·25 ml. volumes and incubated either at 37° C. for 1 hr. or overnight at 4° C. After incubation, 0·5 ml. of cells was added. Of the three methods of treating sera, acetone extraction appeared to be the best. From the limited trials it seemed that kaolin might reduce the level of specific antibodies. Overnight incubation at 4° C. produced better results than those observed with incubation at 37° C. for 1 hr.

Table 1

Serum No.	CF titres (reciprocal of initial serum diluted with 4 units of antigen Indian strain)	Log neutralization index*	HI titres (reciprocal of initial serum diluted with 4 units of antigen Indian strain)
603360–3	32	3·1 to $\geqq$ 3·5	40–160
603366–5	8	3·0 to $\geqq$ 3·1	20–80
603381–4	32	2·5 to $\geqq$ 3·7	160–640
603390–3	16	$\geqq$ 3·5	80–320
603661–2	8	2·2 to $\geqq$ 4·0	20–80
603665–2	32	2·7 to $\geqq$ 4·0	40–160

* Adult mouse IC test with several Indian strains, using constant serum-varying virus dilutions.

CF, Complement-fixation ; HI, hæmagglutination-inhibition.

A group of horse sera collected during a recent epidemic in India were tested for hæmagglutination-inhibition. Six sera that did not show detectable complement-fixing and neutralizing antibodies were also negative for hæmagglutination-inhibiting antibodies. Table 1 compares the complement-fixation, neutralization and hæmagglutination-inhibition results of the remaining six sera. The hæmagglutination-inhibition pattern, while clearly indicating inhibition, is not yet entirely satisfactory. The end-point is not as sharply defined as in tests with other viruses but is spread over several tubes. For this reason the hæmagglutination-inhibition titres have been presented as ranging over several tubes.

McIntosh[2] has reported that the complement-fixation test is less type-specific than the neutralization test and that no antigenic differentiation between the heterotypic strains was possible. Work is in progress to determine if the hæmagglutination-inhibition test is type-specific.

I wish to thank Drs. Tara B. Gokhale and Keerti V. Shah for carrying out the complement-fixation and neutralization tests.

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GENETICS

Developmental Barriers in a Selection Experiment

The present experiments describe the effect of selection on expression of the *ocelli-less* mutant, sex-linked recessive, affecting the ocelli and three pairs of macrochætes on top of the head in *Drosophila subobscura*.

In the foundation population, homozygous for the *ocelli-less* mutant, a large number of flies lacked all 3 ocelli and all 6 bristles ; in the rest, varying combinations of bristles and ocelli were present. The ocelli in some of the flies were found to be slightly displaced from their normal positions, and in certain cases their sizes were also affected. In a few flies the bristles were found to be repeated ; instead of one normal bristle on one side, additional bristles were found to lie close to the normal bristle. A positive correlation ($r = 0·46$) was found between the presence of bristles and of ocelli. Therefore, in measuring the degree of expression, a unit score was given for the presence of each of the 6 bristles and 3 ocelli. Thus a fly completely lacking ocelli and bristles would have a total score of zero and a wild-type fly a total score of 9. No addition to the total score was made for repeated bristles; but their frequency was recorded in the foundation population and in the last few selected generations. The word 'score' will be used throughout in the sense here defined.

In the foundation population the mean score of females was 1·76 and of males 1·44; the sex difference, although small, was found consistently in all the cultures scored.

Directional selection was effective in increasing the mean number of ocelli and bristles almost to the wild-type score of 9 in the upward direction, and decreasing the score to zero in the downward direction. Both the variance of the score and the sex difference in score were greatest in populations the mean score of which was at the centre of the range.

The distribution of the phenotypes in the selected populations suggested that there exists a developmental barrier which prevents progress beyond the upper and lower bounds. These bounds were sharper in the downward direction because other bristles were rarely affected by the mutant. The flies, however, after reaching the score of zero, showed a notch-like depression on the lower margin of the head, which varied in the degree of expression. In the upward selected population, in the last generations of selection, a few flies with extra ocelli were seen, and the frequency of repeated bristles was found to be considerably greater than that in the foundation population.

In Fig. 1 a genetic model is shown to explain the bounded distribution of phenotypes, in terms of genes which in their primary effects on an ocelli-bristle-forming substance are additive throughout the range.

In Fig. 1*a* it is supposed that there are a number of pairs of alleles which alter the expression of the *ocelli-less* flies by altering the amount of some 'precursor' which is necessary for the formation of ocelli and bristles, and which is normally present in the required amount in wild-type flies having the + oc gene. In *ocelli-less* flies the amount of this precursor is variable, but usually less than in flies carrying the +oc gene. Now if the amount of the precursor is less than T_1, no ocelli or bristles are formed. If it is between T_2 and T_3 the wild-type score of 9 is produced. Flies having amounts of precursor intermediate between T_1 and T_2 tend to produce intermediate scores. Only flies having the amount of precursor greater than T_3 produce a score greater than 9. That flies with an amount of precursor within the range T_2–T_3 should all have the same phenotype of 9 is to be expected from Waddington's argument concerning canalization[5]. The amount of precursor in flies carrying the +oc gene is unlikely to be absolutely constant. If we suppose, as shown in the diagram, that +oc flies have an amount of precursor within the range T_2–T_3, then canalizing selection during the past evolution of the species would ensure that the same adult phenotype would develop provided that the amount of precursor is within this range.

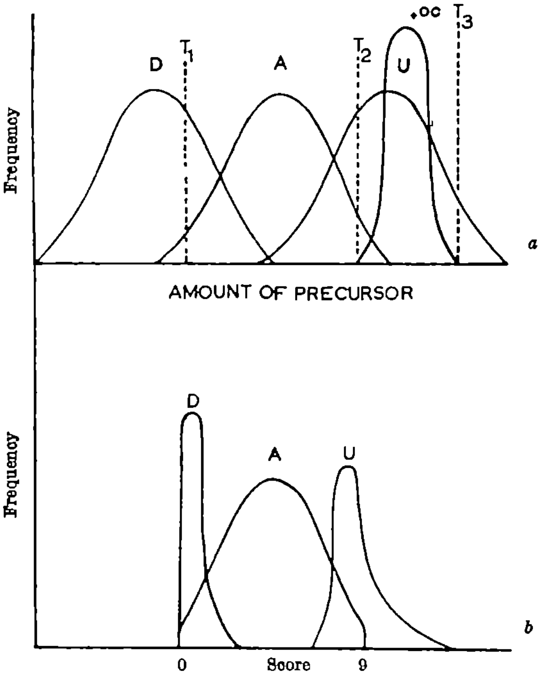

Fig. 1. Model to explain the bounded distribution of phenotypes

The three populations *A*, *D* and *U* in Fig. 1*b* represent, respectively, the phenotypes of a population with a mean score at the centre of the range and downward and upward selected populations. In *A* the genetic variance of the score is largely additive, but in populations *D* and *U* the genetic variance of the score is largely epistatic.

The concept of the 'amount of precursor' has been introduced to facilitate the discussion. A similar model could be given if it were assumed that the different scores are determined by differences in the competence of cells to respond to an unvarying pre-pattern[4].

On the basis of this model most of the experimental results can be explained satisfactorily. A similar model has been suggested by Rendel to explain the results of selection on the scute phenotype in *Drosophila*[2]. However, the situation is complicated by the fact that in some of the flies repeated bristles or bristles at new sites were seen, even in the absence of other bristles or ocelli, suggesting that the flies are abnormal not only in the amount of the precursor but also in its distribution. The main aim of the model, however, is to show how a bounded distribution of adult phenotypes with epistatic genetic variance could be the result of a character, which, if measured earlier in development, is continuously varying and of which the genetic variance is additive.

A more detailed account of this work is given elsewhere[3]. The genetic mechanisms responsible for the distribution of the ocelli-bristle-forming precursor have been described by Maynard Smith and Sondhi[1]. By selecting flies for the symmetrical and asymmetrical patterns of ocelli it has been shown that selection can alter not only the amount of precursor but also its distribution along the antero-posterior gradient, although it cannot modify its distribution along a gradient from left to right.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public*

Monday, January 23

University of London (in the Assembly Hall, Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Mr. J. S. Fulton: "The Development of the Newer Universities".*

British Society for the History of Science (in the Council Room (second floor) of the Science Museum, Exhibition Road, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. H. R. Calvert: "The History of Mathematical Computers".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. H. S. Burhop: "The Techniques of High Energy Physics"* (Inaugural Lecture).

University of London (in the Hatton Lecture Theatre, Queen Mary College, Mile End Road, London, E.1), at 5.30 p.m.—Prof. David C. White (Massachusetts Institute of Technology). "Electrical Energy Conversion".* (Further lectures on January 24 and 26.)

Cement and Concrete Association, Pre-stressed Concrete Development Group (at the Friends House, Euston Road, London, N.W.1), at 6 p.m.—Prof. T. Y. Lin (University of California): "A Review of Advances in Prestressed Concrete in the U.S.A.".

Institution of Mechanical Engineers, Lubrication Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Co-ordination of Experimental Work—an Attempt to Determine whether Lubrication Investigations Carried Out by different Bodies can be Integrated".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. James Taylor: "Towards a Philosophy of the Modern Chemical Industry". (First of three Cantor Lectures on "The Modern Chemical Industry in Great Britain".)

University of London (at Birkbeck College, Malet Street, London, W.C.1), at 6 p.m.—Dr. S. Biesheuvel (Johannesburg): "Problems of Cross-cultural Ability Testing".

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. G. A. Williams: "Economic Heating by Electrode Boilers in Industry".

Royal Institute of Chemistry, London Section (at Enfield Technical College, Queensway, Ponder's End), at 7 p.m.—Prof. R. L. Wain, F.R.S.: "Chemical Control of Plant Diseases".

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. R. C. Rainey: "Locust Control from the Air".

Tuesday, January 24

Institution of Chemical Engineers, South-Eastern Branch (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 3.30 p.m.—Inaugural Meeting.

Institution of Chemical Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. R. Edgeworth Johnstone: "A Survey of Chemical Engineering Education and Practice".

Institution of Electrical Engineers, Measurement and Control Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Machine-Tool Control" opened by Dr. E. H. Frost-Smith.

Parliamentary and Scientific Committee, General Committee (at the House of Commons, London, S.W.1), at 5.30 p.m.—Meeting with Vice-Chancellors of certain universities.

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. R. S. Lenk: "Papers from Non-Cellulosic Fibres"

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. A. A. Fyall, Mr. D. C. Jenkins and Mr. R. N. C. Strain: "Some Problems of Impact and Erosion in Flight".

Wednesday, January 25

Institute of Physics and the Physical Society, Colour Group (at Northampton College of Advanced Technology, St. John Street, London, E.C.1), at 3 p.m.—Mr. F. Pollak: "Some Properties of Coloured Half-tone Images"; Dr. J. Hughes: "Colour Xerography".

Geological Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. L. B. Tarlo: "Middle and Upper Devonian Psammosteids from Scotland"; Dr. W. D. I. Rolfe: "The Geology of the Hagshaw Hills Silurian Inlier, Lanarkshire".

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. W. E. Willshaw: "Generation and Amplification in the Millimetre Wave Field".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. H. Mole: "Radioactive Isotopes and Teeth".* (First of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. W. R. Hawthorne: "The Early Development of the 'Dracone' Flexible Barge" (Thomas Lowe Gray Lecture).

Society of Chemical Industry (joint meeting of the Food Group and Oils and Fats Group, at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Prof. A. C. Frazer: "Some Biological Effects of Heated Fats".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. K. R. McLachlan: "Noise Correlation Measurements".

Society for Analytical Chemistry, Microchemistry Group (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m.—Discussion Meeting on "Hydrogenation Methods in Microanalysis" opened by Mr. R. A. D. Smith.

Royal Institute of Chemistry, London Section (joint meeting with the Slough College Scientific Society, at the Slough College of Further Education, William Street, Slough), at 7.30 p.m.—Prof. R. S. Nyholm, F.R.S.: "Magnetism in Inorganic Chemistry".

British Psychological Society, Medical Section (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 8.15 p.m.—Annual General Meeting, followed by Mr. S. H. Foulkes: "Psychotherapy 1961" (Chairman's Address).

Thursday, January 26

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. Bloor, Mr. T. J. Dean, Mr. G. O. Jones, Mr. D. H. Martin, Mr. P. A. Mawer and Mr. C. H. Perry. "Spectroscopy at Extreme Infra-Red Wave-lengths"; Mr. G. O. Jones, Mr. D. H. Martin, Mr. P. A. Mawer and Mr. C. H. Perry: "The Lattice Resonances of Ionic Crystals".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. E. S. Maurer. "Flowers and Perfumes": Dr. S. M. Manton, F.R.S.. "Evolutionary Paths to Success Among the Millipedes".

University of London (at Westfield College, Kidderpore Avenue, Hampstead, London, N.W.3), at 5.15 p.m.—Prof. E. H. Sondheimer "The Mathematical Description of Nature"* (Inaugural Lecture).

University of London (in the Bearsted Theatre, London Hospital Medical College, Turner Street, London, E.1), at 5.30 p.m.—Dr. F. C. Fraser: "Adaptations in Cetaceans".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. W. V. Mayneord: "Radioactivity of the Human Environment".* (Fifth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. T. Vályi-Nagy (Debrecen, Hungary): "Pharmacology of Antibiotics".*

Institution of Mechanical Engineers, Applied Mechanics Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Informal Discussion on "Applications of Electronic Computers in the Study of Applied Mechanics and Electronic Computing in Engineering Design".

Friday, January 27

Institution of Mining Engineers (at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 11.15 a.m.—67th Annual General Meeting. Mr. R. A. Moore: Presidential Address.

Institute of Rural Life at Home and Overseas (at Friends International Centre, 32 Tavistock Square, London, W.C.1), at 1.15 p.m.—Dr. Wallace Aykroyd: "The Work and Objectives of F.A.O.".*

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. P. B. Medawar, F.R.S.: "Principle and Paradox in Eugenics" (The Woodhull Lecture).

Saturday, January 28

Biochemical Society (in the Courtauld Hall, Queen Elizabeth College, Campden Hill Road, London, W.8), at 10.25 a.m.—402nd Meeting.

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Miss F. Hall: "Animal Courtship".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant, Grade B (with a good honours degree in physics) in the Department of Physics and Mathematics—The Principal, College of Further Education, Flatt Walks, Whitehaven, Cumberland (January 27).

Assistant (with some knowledge of fresh-water biology and chemistry) in the Fisheries and Pollution Department, for duties which will include the inspection of sewage works and trade waste treatment plants, river surveys, and the examination of samples.—The Chief Engineer, Somerset River Board, The Watergate, West Quay, Bridgwater, Somerset (January 30).

Assistant Lecturer in Physics—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (January 31).

Assistant Lecturer or Lecturer in Organic Chemistry—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (January 31).

Examiner (with a first- or second-class honours degree (or equivalent) in chemistry and two years experience in chemistry) of Applications for Patents—The Secretary, Civil Service Commission, 45 Upper O'Connell Street, Dublin, Republic of Ireland (January 31).

LECTURER (medical graduate, with experience in virology and in research methods) IN BACTERIOLOGY—The Secretary, The University, Edinburgh (January 31).

LECTURER or ASSISTANT LECTURER IN INORGANIC CHEMISTRY—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (February 3).

RESEARCH WORKER (graduate, familiar with numerical methods and computers), to study calculations performed in the shipbuilding industry and to construct programmes for an automatic computer—The Director, University Computing Laboratory, 1 Kensington Terrace, Newcastle upon Tyne 2 (February 4).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CIVIL ENGINEERING or PHYSICS—The Registrar, Trinity College, Dublin, Ireland (February 12).

PROFESSOR OF MATHEMATICS (EITHER PURE OR APPLIED)—The Registrar, The University, Leeds 2 (February 14).

READER IN EXPERIMENTAL PATHOLOGY at the Institute of Cancer Research, Royal Cancer Hospital—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 16).

HEAD (with good academic qualifications, with teaching and/or industrial experience) OF THE DEPARTMENT OF APPLIED CHEMISTRY—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1 (February 17).

LECTURER (with at least a good honours degree in geography, and preferably with experience of teaching geography in a university department) IN GEOGRAPHY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth 36 Gordon Square, London, W.C.1 (Hong Kong and London, February 17).

RESEARCH FELLOW IN THE DEPARTMENT OF PHYSIOLOGY, John Curtin School of Medical Research, Australian National University, to work in the field of neurophysiology—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 17).

READER IN MICROBIOLOGY at Queen Elizabeth College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 21).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOW IN BIOCHEMISTRY, CHEMISTRY, ENGINEERING, PHARMACOLOGY or PHYSICS or related subjects—The Secretary, The University, Aberdeen (February 25).

ASSISTANT LECTURER (honours graduate) IN PHYSICS; and ASSISTANT LECTURERS (2) (honours graduates) IN BOTANY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (February 28).

ASSISTANTS (2) ONE IN MATHEMATICS and ONE IN STATISTICS—The Secretary of University Court, The University, Glasgow (February 28).

RESEARCH FELLOW IN MATHEMATICS—The Secretary of University Court, The University, Glasgow (February 28).

SENIOR LECTURER (with high mathematical qualifications and preferably previous experience in working with a digital computer) IN MATHEMATICS at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 28).

LECTURER IN PHILOSOPHY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 4).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY or PHYSICS—The Registrar, The University, Hull (March 7).

LECTURERS (2) IN ENGINEERING MATHEMATICS—The Secretary, The Queen's University, Belfast, Northern Ireland (March 10).

LECTURER (with an honours degree in metallurgy or a related subject, or with equivalent qualifications) IN CHEMICAL AND PROCESS METALLURGY at the Wollongong Division (University of New South Wales)—The Agent-General for New South Wales, 56 Strand, London, W.C.1, and the Appointments Section, University of New South Wales, Box 1, Post Office, Kensington, N.S.W., Australia (March 30).

ASSISTANT DIRECTOR (with a good science degree, preferably with some knowledge of genetics and/or statistics, and a knowledge of at least two foreign languages) OF THE BUREAU OF ANIMAL BREEDING AND GENETICS, King's Buildings, West Mains Road, Edinburgh 9—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (March 31).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMOTHERAPY, ENGINEERING, METALLURGY, PHARMACOLOGY or PHYSICS—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 15).

ASSISTANT BIOCHEMIST (with a first- or second-class honours degree in chemistry or biochemistry) IN THE AREA PATHOLOGY LABORATORY based at Stoke Mandeville Hospital—The Administrative Officer, Stoke Mandeville Hospital, Aylesbury.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree in zoology or bacteriology and preferably experience in serology), for work in the Serology Section—The Secretary, Research Institute, Pirbright, Surrey.

ASSISTANT or ASSOCIATE PROFESSOR (with an honours degree in mathematics and preferably some postgraduate experience in research or teaching) IN THE DEPARTMENT OF MATHEMATICS—Head, Department of Mathematics, University of New Brunswick, Fredericton, N.B., Canada.

CHAIR OF PHARMACY—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1.

CHEMIST (with a first- or second-class honours degree in chemistry or equivalent qualification, and preferably experience in the inorganic analytical field and a knowledge of geochemical techniques) in Nyasaland, to take charge of the Geological Survey Department's chemical laboratory and to undertake assaying and chemical testing of rocks, including full silicate rock analyses—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.105/4/04.

EXPERIMENTAL OFFICERS (2) (graduates in physics or chemistry and interested in research in the field of high temperature gas dynamics), for plasma jet research—Head of the Aeronautics Department, Imperial College of Science and Technology, London, S.W.7.

PHARMACOLOGIST, BIOCHEMIST or ORGANIC CHEMIST with biologica experience (Ph.D. standard), for work in the field of chemotherapy—The Secretary, Medical Research Council of Ireland, 50 Merrio Square, Dublin, Republic of Ireland.

RESEARCH ASSISTANT (preferably graduate, with laboratory experience in chemistry or biochemistry), for work on amino-acid metabolism—Dr. L. I. Woolf, Department of Regius Professor of Medicine Radcliffe Infirmary, Oxford.

SENIOR SCIENTIFIC OFFICER (with a good honours degree in on of the following: agriculture, chemistry or biochemistry, with a least two years research experience), to act as Secretary of a propose Research and Development Committee which will be concerned with problems relating to the growing, harvesting, handling and marketing of potatoes—Establishment Officer, Potato Marketing Board 50 Hans Crescent, Knightsbridge, London, S.W.1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The British Cast Iron Research Association. Thirty-ninth Annual Report for the year ending 30th June, 1960. Pp. 55. (Alvechurch, Birmingham: British Cast Iron Research Association, 1960.) [2410

Association of Universities of the British Commonwealth. United Kingdom Postgraduate Awards, 1960–62: Fellowships, Scholarships, Grants, etc., tenable at universities in the United Kingdom. Short list of awards tenable outside the United Kingdom. Tenth edition. Pp. ii+136. (London: Association of Universities of the British Commonwealth, 1960.) [2610

Scientific Council for Africa South of the Sahara. CSA/CCTA. Publication No. 52: CSA Meeting of Specialists on Open Forests in Tropical Africa, Ndola, Northern Rhodesia. Pp. 126. (London. Bureau of Publications, CCTA/CSA Secretariat, Watergate House, York Buildings, W.C.2, 1960.) [2610

Colonial Office. Report of the Public Services Conferences held in the Colonial Office, London, 1st to 10th March, 1960. Pp. iii+25. (Colonial No. 347). 3*s*. 6*d*. net. Service with Overseas Governments. Pp. 22. (Cmnd. 1193). 1*s*. 3*d*. net. (London: H.M. Stationery Office, 1960.) [2710

Other Countries

Ceylon. Administration Report of the Director of National Museums for 1959. Pp. 51+3 plates. (Colombo: Government Publications Bureau, 1960.) Rs. 1.50. [241 0

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 16, No. 7: Fertility Cult: U.S.A. Pp. 133–150. (Washington, D.C.: Population Reference Bureau, Inc., 1960.) 50 cents. [2410

Tea Research Institute of East Africa. Annual Report for the year 1959. Pp. 40. (Kericho: Tea Research Institute of East Africa, 1960.) [2410

Proceedings of the California Academy of Sciences. Vol. 30, No. 1 (September 30, 1960): Investigations in the Natural History of Baja California. By Ira L. Wiggins. Pp. 1–45+4 plates. (San Francisco California Academy of Sciences, 1960.) [2410

United States Department of the Interior: Geological Survey. Professional Paper No. 400–A: Geological Survey Research 1960—Synopsis of Geological Results. Pp. xii+136. Professional Paper 400–B: Geological Survey Research 1960—Short Papers in the Geological Sciences. Pp. viii+515. 4.25 dollars. (Washington, D.C. Government Printing Office, 1960.) [2410

Chicago Natural History Museum. Fieldiana: Anthropology. Vol. 51, No. 2: Table Rock Pueblo, Arizona. By Paul S. Martin and John B. Rinaldo. Pp. ii+131–298. 5.50 dollars. Fieldiana: Geology. Vol. 14, No. 2: A New Specimen of *Desmatochelys lowi* Williston—a Primitive Cheloniid Sea Turtle from the Cretaceous of South Dakota. By Rainer Zangerl and Robert E. Sloan. Pp. 7–40+2 plates. 1 dollar. Fieldiana: Geology Memoirs. Vol. 3, No. 5: The Vertebrate Fauna of the Selma Formation of Alabama. Part 5: An Advanced Cheloniid Sea Turtle. By Rainer Zangerl. Pp. ii+281–361+plates 30–34. 3.50 dollars. Vol. 42, No. 7: Philippine Zoological Expedition, 1946–1947. Camptopterohelea—a New Genus of Ceratopogonidae from the Philippines. By W. W. Wirth and A. A. Hubert. Pp. 89–91. 10 cents (Chicago: Chicago Natural History Museum, 1960.) [2410

Jamaica. Banana Board Research Department. Occasional Bulletin No. 2: Breeding the Immune Banana. By E. E. Osbourne. Pp. 8. (Kingston: Government Printer, 1960.) [2410

Jamaica: Ministry of Agriculture and Lands. Fifty Years of Cattle Breeding and Development. Pp. 16. Annual Report of the Ministry of Agriculture and Lands for the year ended 31st December, 1958. Pp. 92. 4*s*. (Kingston: Ministry of Agriculture and Lands, 1960.) [2410

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EMPLOYMENT OF WOMEN SCIENTISTS AND ENGINEERS

THE report of an inquiry conducted by the Social Survey of the Central Office of Information into the employment of qualified women scientists and engineers in private manufacturing industry shows clearly that, in general, industry in Britain is a man-dominated world and is likely to remain so for many years to come*.

The sample of establishments covered by the survey was weighted in favour of industries which employ a high proportion of qualified scientists and engineers, and was drawn from establishments with two hundred workers or more which in 1956 were known to employ scientists and engineers. The number of establishments which took part in the survey was 785, employing between them 16,350 qualified scientists and engineers, that is, about 25 per cent of all those engaged in manufacturing industry.

Of the co-operating establishments, only about 98 (13 per cent) employed women scientists and engineers at the time of the survey. A further 85 (11 per cent) establishments had employed women scientists and engineers in the past but did not do so at the time of the inquiry. The 785 co-operating establishments employed between them 15,972 male and 378 female qualified scientists and engineers ; that is, 42 qualified men for every qualified woman. Only eight of the 378 women were engineers. On the assumption that the ratio of scientists to engineers in the sample was the same as in manufacturing industry as a whole, the ratio of male to female scientists was fifteen to one, and of male to female engineers more than a thousand to one.

An interesting feature of the inquiry was the views put forward by managers indicating their reasons for not being enthusiastic about the employment of qualified women scientists and engineers. Three main disadvantages were repeatedly mentioned, namely, that women were too likely to leave because of domestic commitments, including marriage ; that there were difficulties about having them in authority over, or working with, male personnel ; and that rough, dirty and physically hard working conditions were unsuitable for women.

Despite all the pleadings put forward by women's organizations, these are cogent points to support views strongly held by cost-conscious industrial managements. The training of scientific and technical personnel for responsible positions is an expensive process, and the facilities are much more readily given to those who are likely to stay with firms than those who are likely to go.

Qualified women are unlikely to become acceptable in industry until the shortage of technical personnel has become so acute that employers are prepared to accept them although fully aware of the fact that they are much more likely to have left within a few years than members of the opposite sex. Not all the views expressed by management, however, can be substantiated. Far too much is made of the case that male staffs object to having women in authority over them. In the Social Survey inquiry, for example, there was considerable difference between the views of those managements which had employed women scientists and those which had never employed them. Where there was experience of employing women scientists, it was much more often held that the male staff were in favour of their employment or had no objection to it.

Not unexpectedly, there is far greater objection to the employment of women engineers than women scientists. The conditions under which professional engineers often have to work, for example, dealing with heavy and dirty machinery, plant and site work, and shift work, are regarded as unsuitable for women while the control of male labour in engineering organizations would be possible only for the most exceptional women engineers. It is significant that very few employers in the survey had had any actual experience of employing women engineers.

In many ways the second part of the survey is likely to be of greater use in enabling educated women to find a suitable niche in industry. It was concerned with the actual experience of women who had taken university degrees in science or engineering in recent years. The sample taken was one in two of all women scientists who had graduated in 1954 and 1956. These years were chosen because women who graduated in 1954 could still be traced and would have had at least four years in which to gain experience of employment in industry and elsewhere. Those who had graduated in 1956 would have had only a little more than two years employment: but it was felt to be worth while to find out whether the situation had changed in recent years.

Of the 708 women in the sample, 381 were unmarried, 214 were married without children and 133 were married with children. The number working full-time was 513 (73 per cent), of whom all but 7 were single or married without children. Thirty-five were working part-time and 160 (22½ per cent) were not in paid employment. All except 31 (4 per cent) had had a paid job since graduation. Of the single women in the sample, 92 per cent were working full-time, and, of the married women without children, 73 per cent were working full-time.

Of the graduates who were still working full-time at the time of the survey (513), about half of them were, or had been, employed in teaching. The next largest proportions were engaged in research and development, and testing, analysis and product control, which bears out the finding in the employers' survey that these were the jobs in industry in which women scientists were most often engaged. 74 per cent of the graduates were employed in professional services, only 19 per cent in the industries included in the employers' survey, and 7 per cent were in other businesses. The professional services include education, medical and hospital services, and trade and professional associations; other businesses include gas, electricity and water, transport, national and local government, insurance, banking and finance, and catering.

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* *Ministry of Labour Gazette*, September 1960.

In general, graduates working in private manufacturing industry were earning more than those in the professional services and in other businesses. Thus, of the graduates in private industry, 39 per cent were earning £800 or more per annum compared with 23 per cent in the professional services, and 30 per cent in other businesses. Because of the high proportion of graduates employed in teaching, the level of salaries shown as paid in the professional services must have been greatly influenced by the lower salaries in the teaching profession.

Women science graduates were asked whether their jobs offered prospects of promotion and whether, in their view, male colleagues were given preferential treatment. Of those employed in manufacturing industry more than half (52 per cent) thought that there was no prospect of promotion, and rather less than half (42 per cent) believed that there was. In the professional services, which included teaching, these proportions were reversed. In the 'other businesses' category a much higher proportion of women (69 per cent) thought that there were prospects of a better job.

More than half the women scientists employed in private manufacturing industries thought that their employers showed bias in favour of men as regards pay and promotion, but only 16 per cent in the professional services thought so. There were many in the latter group, however, to whom the question did not apply because all their colleagues, for example, in girls' schools, were women.

From both parts of the survey one conclusion is inescapable. Employers are reluctant to employ educated women scientists and engineers mainly because, on economic grounds, they are a bad risk; the present preponderance of eligible males over females in the population means that less and less females are avoiding marriage. Changing social behaviour has also led to a marked reduction in the age at which marriage is contracted; and, although the majority of newly married women are staying at work for some time after marriage, the age at which they bear their first child is also being reduced. From the employer's point of view, their years of useful service before beginning full-time duties in their homes is very limited. All the well-meaning protestations by women's organizations will not make young women scientists and engineers anything but a bad industrial investment compared with their male counterparts. Most educated women know this and accept it.

Although this view may not be openly expressed by employers, or may be hidden by plausible excuses about women's inability to control male staffs or put up with bad working conditions, its force is so strong that there are to-day more vacancies for women scientists in industry than there are women available to fill them. In spite of the greater financial attractions of industry, qualified women turn to careers where they feel wanted, where there are greater opportunities for positions of responsibility and promotion, and, above all, where they are likely to be acceptable again in their early forties when their child-bearing and home-making functions may have finished. For this reason, and because of the difficulty of keeping up to date in science and technologies which demand continuous reading, discussion and practice, women are still looking for careers in teaching, medicine, catering, personnel management and those branches of the professional and social services where the 15–20 years spent in raising a family may prove not a handicap but an asset. It is to be hoped that, on their return to employment, responsible authorities will recognize the added maturity and experience which marriage has brought and, after an appropriate settling-down period, see that they are not debarred from opportunities of promotion.

PAUL EHRLICH'S COLLECTED PAPERS

The Collected Papers of Paul Ehrlich

Compiled and edited by Dr. F. Himmelweit, with the assistance of the late Martha Marquardt. Under the editorial direction of Sir Henry Dale. Vol. 2: Immunology and Cancer Research. Pp. viii+562+9 plates. Vol. 3: Chemotherapy. Pp. viii+615+6 plates. (London and New York: Pergamon Press, 1960.) 160*s*. net each.

WITH these two volumes practically all the publications of Ehrlich alone or jointly are concluded. Volume 2 contains research on immunity. This work really started with the immunization of animals against plant toxins (ricin, etc.) and showed that, in analogy with Behring and Kitasato's diphtheria antitoxin, a specific antiricin appeared in the blood serum. Further, the important discovery was made that its action could be demonstrated *in vitro* as well as *in vivo*. Experience so gained was then applied to the practical problem of assessing quantitatively the antitoxin content of antidiphtheria sera. Besides finding a solution, Ehrlich also formulated the famous side-chain or receptor theory to explain the mechanism of antibody formation in general. This embodied a conception already presented in his "Sauerstoff Bedurfniss". The work was entitled "Die Wertbemessung des Diphtherieheilserums" (a translation is supplied). Further studies on immunity, especially with Bordet's hæmolysins, led to a series of papers, which were re-issued together as "Gesammelte Arbeiten zur Immunitätsforschung". In the preface Ehrlich states that the volume shows how his theory of immunity is "essentially the summary of an extraordinarily large number of exact investigations". However, nearly two-thirds of the papers does not bear

Ehrlich's name and so is omitted here, as is the case also in other sections. This must be understood, otherwise readers might infer that *The Collected Papers* contains all the work of the school inspired and directed by Ehrlich. Doubtless the matter exercised the editors; further inclusions certainly would have weighted the enterprise heavily. Of course, he usually quoted his co-workers' results, since they contributed to support Ehrlich's theories, which were indeed "all his very own". But occasionally some finding was documented which had no significance in this context, although afterwards found to be important in quite another connexion. The side-chain theory, in so far as it identifies antibody formation with normal processes of cellular metabolism, has not been superseded. But various features have required revision, for example, the amboceptor hypothesis. Also, the production of auto-antibodies and their pathogenic effects are now recognized as occurring widely, while factors which may antagonize antigenicity (immune tolerance) or influence allergy are gaining prominence. The experimental work on cancer by Ehrlich and Apolant, also in Volume 2 (but only a part of the Institute's publications in this field), was invaluable in establishing this line of research. But Ehrlich's nutritional theory of athrepsy to account for certain phenomena of tumour-growth has not yet produced striking developments.

Volume 3 comprises the chemotherapy of infections. The original essay (1891) was the successful treatment with methylene blue of two cases of indigenous quartan malaria, this dye being suggested apparently by its strong affinity for the malarial amœba. But thirteen years passed before systematic investigation on chemotherapy in experimental trypanosomiasis began—that with Shiga, on trypan-red. In 1906 both biological and chemical work on chemotherapy was provided for in the new Georg-Speyer-Haus. Ehrlich's inaugural address, here published for the first time, related the origin of his interest in chemotherapy and long-cherished wish to devote himself to it, at last fulfilled. The first paper from the Speyer-Haus (1907) describes most of the phenomena of chemotherapeutic biology, which have since been very widely encountered with the various drugs, parasites and hosts investigated. One misses an English version of those two classics. Incidentally, an interesting light can be thrown on Ehrlich's attitude to matters of publication by a fact which does not come within the range of *The Collected Papers*, namely, that chemotherapeutic interference, discovered at the Speyer-Haus and demonstrated to him in 1907, was never published in his lifetime (Browning, *Ann. N.Y. Acad. Sci.*, 59, 204; 1954). More than one-third of the volume is occupied by the salvarsan drugs. Ehrlich's principle, "corpora non agunt nisi fixata", which pervaded his outlook on immunity, also inspired his views on chemotherapy—drugs which act on pathogenic organisms must combine with the latter through chemoceptors, which, however, have not yet been defined chemically. An addendum consists of tributes on the deaths of Ehrlich's intimate scientific associates, Weigert (his cousin), Albrecht, Koch, Bertheim and Apolant.

At last Ehrlich's theories and descriptions of his work in his own telling language are all easily accessible, with versions of a proportion of them also in English. Sir Henry Dale has introduced each volume with his brief and lucid surveys and perspectives. Many glosses also testify that the editors have performed their heavy self-imposed duty with high seriousness. They have aimed to ensure that Ehrlich's enormous versatility and range of understanding alike in biology and chemistry, the numerous departments of knowledge which he either created or immensely enriched and the genius immanent in his contributions both to science and practice, shall be conspicuous.

C. H. Browning

CALORIFIC VALUE OF GASEOUS FUELS

Gas Calorimetry

The Determination of the Calorific Value of Gaseous Fuels. By C. G. Hyde and M. W. Jones. Second edition, entirely revised and reset. Pp. vii+456. (London: Ernest Benn, Ltd., 1960.) 105*s*. net.

The subject of gas calorimetry is of great importance to the gas industry, both for the proper assessment of its thermal efficiency and to enable it to comply with its statutory obligations. Since 1932 the standard work on the subject has been "Hyde and Mills", and in view of developments within the past twenty-five years the appearance of a second edition, in which Mills's part is taken by M. W. Jones, is to be welcomed.

While the original plan of the work has been retained, the book has been largely re-written. Major Hyde was until recently controller of gas standards, and Mr. Jones is principal gas examiner under the Ministry of Power, so that the authors are well qualified to deal with their subject, and *Gas Calorimetry*, 1960, may be taken as a full, modern and authoritative exposition.

It is useful to find the fundamental theory of calorimetry and thermometry combined with its application to specific instruments in a book which, being thus broadly based, has educational as well as practical value.

The new edition welcomes fluorescent lighting for test rooms, and describes a mobile unit of value in a scattered area of supply. It considers the Hyde meter prover, and might perhaps have omitted the older and largely obsolete Harcourt prover. The Mark II Fairweather recorder is well described, though it is difficult for publication to keep up with development in this sphere. It is particularly useful to have a clear description of the Reineke calorimeter, which is of increasing interest now that liaison between the gas and oil industries is becoming so close. The very useful set of tables in the appendix has been extended, and the placing of references at the end of their appropriate chapters is an advantage.

There is a brief consideration of the combustion characteristics of gas, but in view of the growing importance of this subject a fuller treatment would have been of interest, and some description of modern methods for the direct recording of the Wobbe index would have been valuable.

There is still a good deal of descriptive matter about obsolete or obsolescent instruments, even when one allows for the valuable principles sometimes involved. Surely no one to-day uses—if indeed they ever successfully used—a Lupton water heater. Moreover, the devotion of forty-one pages to the Boys recording calorimeter, ingenious as it was and distinguished as was its inventor, seems rather excessive. Flame indicators, too, cannot be advanced seriously in 1960 as other than highly misleading instruments. One might also make a plea for the substitution of a more descriptive name than the time-honoured "Tabular Numbers" for gas volume reduction factors. After all, most numbers come from tables.

These are, however, minor criticisms. The format of the book is clear and convenient, and, with the exception of one or two poorly reproduced photographs and an illegible table on p. 74, the printing is excellent. "Hyde and Jones" is altogether a worthy successor to "Hyde and Mills" and will long form the indispensable vade-mecum of all who, whether from the official or works-control angle, are concerned with the measurement of the calorific value of gaseous fuels. Physicists unconnected with the gas industry will also find in it much of value, and if they are surprised to find that an apparently simple operation is fraught with so many pitfalls they will also find the solution to many of their gas calorimetric problems within its pages. R. J. H. BANKS

QUANTITATIVE MORPHOLOGY

Quantitative Zoology

By George Gaylord Simpson, Anne Roe and Richard C. Lewontin. Revised edition. Pp. vii+440. (New York: Harcourt, Brace and Company, 1960.) n.p.

THE first edition of this work was published twenty-one years ago at a time when zoologists, particularly those interested in morphology and systematics, rarely employed statistical methods. In the meantime, interest has tended to shift in many subjects from the individual to the population, with the consequence that the use of statistical methods is much more necessary. The original authors have been joined by Dr. R. C. Lewontin in producing a revised version which, while having the same general plan, is more lucid and has had some mistakes removed. A chapter on the analysis of variance has been added, and the methods given usually assume that a calculating machine is available. The text is essentially a detailed explanation of those elementary statistical methods which are likely to be useful to zoologists in general and taxonomists in particular. Many examples, mostly drawn from the vertebrates, are fully worked out so that no other statistical source would be required. The least mathematical of zoologists should be able to follow the arguments and thereafter apply the methods to his own work. The biological significance of parameters or tests of significance is indicated wherever it is known. The last two chapters deal with graphic methods and with the problems of growth, respectively.

The authors admit that there are now many more good books on biometry, statistics, and general quantitative methods than when this work was first published. The most cogent argument for a new edition was that the book was still in demand though out of print. It is also true that many statistical works, so far as they touch on biology, are biased in the direction of agriculture. It seems debatable, however, whether, in view of the large number of statistical texts available, it might have been possible to reduce this side of the work and to expand it in other directions. The subject of sampling in the actual circumstances of Nature is rarely adequately discussed, and yet it raises many difficulties, though perhaps more for the ecologist. The paragraph on the capture–recapture method is perhaps too short to be useful in view of the real difficulties in interpretation and the many possible modifications of the simplest theory. Possibly the negative binomial distribution might be worth discussing; at least the bibliography might include more references to works in which additional or more refined methods are described.

The great merit of the work as it stands is its lucidity, which in this particular subject is a transcendent advantage. Nearly all taxonomists will find something useful to them in one chapter or another and very little that they cannot understand. O. W. RICHARDS

SULPHATED POLYSACCHARIDES

Polysaccharides in Biology

Transactions of the Fourth Conference, May 21, 22, and 23, 1958. Edited by G. F. Springer. Pp. 326. (New York: Josiah Macy, Jr. Foundation, 1959.) 5.95 dollars.

THIS is the report of a conference held at Princeton, New Jersey, in May 1958 on the sulphated polysaccharides. There are seven chapters, each associated with a main speaker whose account is followed by a discussion and further contributions. The chapter headings are: chondroitin sulphates (K. Meyer), biology of chondroitin sulphates (R. H. Follis), heparin and related substances (M. L. Wolfrom), the mast cell (R. D. Higginbotham), biosynthesis of sulphated polysaccharides (H. Boström), synthetic non-biological sulphated polysaccharides (M. J. Shear) and sulphated polysaccharides of plants (L. Stoloff).

The style of the book is provocative, and there will not be many neutral opinions about it. As in other Macy 'multiprofessional conferences', the proceedings are reported verbatim, with a few afterthoughts interpolated into the text. To this reviewer the presentation, reading like a playscript, has many merits, but a few 'stage directions' in the way of sub-headings would be helpful. There are no sub-headings within the chapters, and, since these are up to 60 pages long, the index assumes a vital function, but even though this is 20 pages long, in the circumstances it is barely adequate. Moreover, the lack of subdivision makes it difficult to discover when the main speaker has finished, and the contents page gives no inkling that a second speaker may also have presented a lengthy description of his own work. In the chapter associated with Meyer's name, the main speaker in terms of continuous matter is in fact Jeanloz. A further general disadvantage is that this is a conference of experts, and definitions which would be out of place in the conference room are necessary to the less-specialized reader. A glossary would be helpful.

An advantage that could be legitimately expected of this type of presentation is that it should be available for printing as soon as the tape-recorder has yielded its secrets to the typewriter. It is surprising to learn from the publishers that 20 months elapsed between the conference and the book becoming available in January 1960. Much that was new at the conference has since been published elsewhere. Nevertheless, the authoritative nature of the papers and the importance of the subject will ensure for the book a wide sale and circulation, and the Macy Foundation is to be congratulated on its timely choice of the subject. The book is well-produced, attractively priced and contains very few errors.

W. J. WHELAN

POLYPEPTIDES ACTING ON SMOOTH MUSCLES

Polypeptides which affect Smooth Muscles and Blood Vessels

Proceedings of a Symposium held in London on 23rd and 24th March, 1959. Edited by M. Schachter. (Biological Council's Co-ordinating Committee for Symposia on Drug Action Series, Vol. 4.) Pp. xv + 336. (London and New York: Pergamon Press, 1960.) 50*s*. net.

THE Co-ordinating Committee for Symposia on Drug Action, under the chairmanship of Sir Charles Harington, has sponsored discussions at the Wellcome Building in London in the spring since 1955. The subjects of these symposia have been histamine, hypotensive drugs, 5-hydroxytryptamine, and quantitative methods in human pharmacology. The present book deals with the fifth of these symposia, and shows how much this subject has developed since 1953 when fifteen authors contributed to a meeting in Montreal and afterwards published their contributions in a book (*Polypeptides which stimulate Plain Muscle*. Ed. J. H. Gaddum. Edinburgh: Livingstone, 1955).

The structures of oxytocin and vasopressin were first announced at that earlier meeting; now it is possible to discuss the relation between the structure and action of a number of homologues. Oxytocinase has been estimated with an artificial substrate, and a separate vasopressinase has been found in the placenta. Substance *P* resembles these pituitary hormones in that it appears to be stored in nervous tissue, but its structure and function are still unknown. The structure of angiotensin was elucidated in 1956, and the actions of a number of homologues have now been measured, but there are still serious doubts about the relation of this substance to hypertensin.

Kallidin, bradykinin and other kinins form a group with very similar actions. The isolation of bradykinin and its composition in terms of amino-acids were announced at the meeting in 1959; the exact structure is now known.

The final session was devoted to anaphylatoxin, leucotaxine, exudin, globulins affecting permeability and the activation of large molecular substances by injury. Some of these latter substances are still not clearly defined and there is much more work to be done by the chemists who have already done so well in this field.

Some of these polypeptides are thought to play important parts in physiology and pathology. Vasopressin controls the excretion of water, and oxytocin controls the uterus and the breast. The kinins may be released to cause vasodilatation in active tissues. There is clearly much to be discovered about all these substances and every reason to expect that they will prove to be physiologically important.

The symposium was a success because it collected the leading authorities from all over the world. Owing to the generosity of the Wellcome Trust and Sandoz, Ltd., it was possible to invite contributors from the United States, Sweden, Switzerland, Germany and Austria.

The book gives an authoritative summary of work in this field. It has been edited by M. Schachter and is beautifully produced, but rather expensive. It did not appear until a year after the symposium on which it is based, but that was partly due to a printers' strike. It is likely to remain the best summary of work in this field for some time to come.

J. H. GADDUM

SEDIMENTARY ROCK TYPES

Microscopic Sedimentary Petrography

By Prof. Albert V. Carozzi. Pp. viii + 485. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 92*s*. net.

THE work of Prof. Albert V. Carozzi is well known to geologists by the publication in 1953 of his *Petrographie des Roches Sedimentaires* and by other important papers. Here he describes his work and that of others in English. Throughout the book the author refers to both American and European examples, and the long list of references at the end of each chapter is by no means the least valuable part of this important book. No attempt is made to confuse the student with a wealth of individual examples, but rather to describe ideal types together with the chief variations. From such descriptions it is a relatively simple matter for the research worker to apply such general principles to his own particular problems.

The author divides the sedimentary rocks into three main groups, namely, the clastic, the biochemical and the chemical rocks. The first and last groups are well known to students, but the middle division is less familiar. Under this heading are described carbonate, siliceous, iron-bearing and phosphoritic rocks which possess the common property of having been generated chiefly by organic as well as chemical processes.

In Part 1 the microscopic characters of the clastic rocks are dealt with under the headings of arenaceous, rudaceous, pyroclastic and argillaceous rocks. Microscopic definitions are reduced to an absolute minimum, and in this as in succeeding sections of the book the author directs attention to the great amount of additional knowledge which is still required for a complete understanding of the genesis of many sedimentary rocks.

Part 2 deals with the biochemical rocks, and an evaluation of the relative value of the two processes —organic and chemical—is attempted.

In the concluding part the evaporites are described. These are, perhaps, among the most difficult of all sedimentary rocks to understand, and the author has wisely not theorized further than published descriptions enable him to do so.

Prof. Carozzi's chief interest has always centred on the carbonates, and the many workers in this field will find much of value in his able summary concerning the formation of the oolites and dolomitization.

The book concludes with extensive author and subject indexes, and is well produced on good paper with a pleasing type, though it is unfortunate that it was necessary to price it rather on the high side.

F. S. WALLIS

Mineral Processing

By Dr. E. J. Pryor. Revised and enlarged edition of "An Introduction to Mineral Dressing". Pp. vi+814. (London: Mining Publications, Ltd., 1960.) 90*s*.

THE second edition of Dr. Pryor's book, under a new title, is a revised, extended and improved version of the first. All the illustrations now have captions, it is less pedantic in places and there are improvements in detail of presentation, but some of the criticisms in the notice of the first edition (*Nature*, 177, 908; 1956) are still valid.

Although, as stated on page 3, "Mathematical presentation has been avoided as far as possible in this book", more formulæ are used in the new edition than in the first, but, when they are given just as formulæ, they do not always help the generally empirical approach (which may lead to undue wordiness) in the way that they should. Such mathematical approaches as are used are spoilt by the haphazard use of symbols. Specific gravity and density (no units given) are used indiscriminately and their symbols may be δ, Δ, ρ, γ, or S. The symbol S is used also for the thickness of the double layer, for entropy, for specific surface and for the 'set' of crushing rolls. The symbol R is used for six different purposes and other symbols have multiple uses—one of them for two purposes on the same page. The formula for Reynolds number appears in two places: in one V is velocity, $2r$ is diameter and δ is density; in the other v is velocity, δ is diameter and ρ is density.

The chapter on the physics and chemistry of pulps has been considerably improved; so has the chapter on coal preparation but perhaps not as much as its importance warrants. The chapter "Gold Cyanidation" has been replaced by a chapter "Chemical Extraction" which devotes 58 pages to gold but only an inadequate 14 to "other metals and minerals".

The book as a whole is well arranged and contains a great deal of information, perhaps too much in connexion with details of process and plant operation, some of which might well be omitted in a new edition and the space so released used for more rigorous mathematical approaches to those problems which lend themselves to such treatment. S. G. WARD

Demineralization by Electrodialysis

Edited by J. R. Wilson. Pp. xv+378. (London: Butterworths Scientific Publications, 1960.) 60*s*.

IN 1959, the world's largest electrodialysis plant came into operation at the Free State Geduld mine in South Africa. This plant, with a design capacity of 2,400,000 gallons of desalted water a day, was the outcome of a programme of research and development in the laboratories of the South African Council for Scientific and Industrial Research. This book by the staff of the Process Development Division of the Council's National Chemical Research Laboratory, is a review of the process of demineralization by electrodialysis, and also a technical record of the various stages in the development and construction of the largest electrodialysis plant which has so far been built. The book contains chapters on the electrodialysis process, the physical chemistry of ion-selective membranes, the preparation and characterization of membranes, the design and operation of electrodialysis plant, pilot-plant operations, and the Free State Geduld electrodialysis plant. The later chapters, which include details of the difficulties encountered in the preparation of the ion-selective membranes and in the construction and operation of the plant, will be of value to all who are concerned with the practical application of electrodialysis to water desalting. The earlier chapters will be of wider interest. The book contains a great deal of useful information and is supplemented by a comprehensive bibliography. The index is a little difficult to use, but otherwise the book is well produced, with very good illustrations and diagrams.

D. K. HALE

On Mathematics and Mathematicians

By Robert Edouard Moritz. (Formerly titled "Memorabilia Mathematica or the Philomaths's Quotation Book".) Pp. vii+410. (New York: Dover Publications, Inc.; London: Constable and Co., Ltd., 1958.) 1.95 dollars; 16*s*.

THE publishers of paper-back reprints are not always happy in their choices, but only the very hard-hearted reader will refuse a welcome to this reprint of the "Philomaths's Quotation Book", long out of print and almost unobtainable. From more than 2,000 quotations we can select informed and ill-informed attempts to define mathematics, we can read what Gauss and Henry Smith have said about the theory of numbers or what Condamine said about the Yancos of the Amazon, whose name for three is 'poettarrarorincoaroac': "Heureusement, leur arithmétique ne va pas plus loin". The Sylvester anecdotes alone are worth the price of the book. An excellent bed-side volume; but it has a deeper value How many of the judgments and opinions which were acceptable in 1914 retain their full worth to-day? If Moritz could give us a 1960 collection of "great thoughts", with quotations from Bourbaki, Brouwer, Hardy, Hogben, Hilbert, Weyl, to name only a few, would the comparison not show a difference in tone and values enough to indicate a profound revolution in our ways of thinking about mathematics? Would it be Trollope against Graham Greene? Theses have been written on less interesting themes.

T. A. A. BROADBENT

Handbook of Geophysics

Revised edition. (United States Air Force/Geophysics Research Directorate.) Pp. xvii+657. (New York and London: The Macmillan Company, New York, 1960.) 105*s*.

BY means of excellent diagrams and detailed tables this volume summarizes our knowledge of that part of geophysics concerned with phenomena above the Earth's surface. Much of the information is intended for the engineer designing aeroplanes and rockets; but the compilation will be of great value to the scientist and of considerable interest to the general reader. For example, the chapter on clouds, which gives tables showing the frequency of various ceiling heights of cloud at various places, provides very interesting reading.

There are chapters on the divisions of the atmosphere, its temperature, density and pressure distribution, a discussion of winds, including data on the jet stream, data on the rain and hail distribution and on the composition of the atmosphere. There are charts of the Earth's magnetic field and data on magnetic activity and atmospheric electricity. Basic information on geodesy is given and thermal and cosmic radiation discussed. The propagation of light, electromagnetic radiation, and sound through the atmosphere is another topic, and there is a very useful chapter on devices for exploring the atmosphere. In places, the writing leaves something to be desired; for example, meteors are defined as "extraterrestrial matter which have penetrated far enough into the earth's atmosphere to become embroiled in a violent reaction with it". S. K. RUNCORN

A Bibliographical Index of the British Flora

Including Floras, Herbals, Periodicals, Societies and References relating to the Identicfiation, Distribution and Occurrence of Phanerogams, Vascular Cryptogams and Charophytes in the British Isles. Compiled by N. Douglas Simpson. Pp. xix+429. (Bournemouth: N. Douglas Simpson, 3 Cavendish Road, 1960.) n.p.

THIS is an ambitious effort to index references concerning the identification, history and geographical range of the flowering plants, vascular cryptogams and charophytes in the British Isles, including Ireland. It also attempts to provide information on plant-lore, local names, poisonous plants and weeds. To accomplish thoroughly such a task would be a prodigious undertaking requiring detailed scrutiny of the vast literature in which records may be expected. It is not surprising, therefore, that the present work is admittedly incomplete, with many publications of local societies and other sources entirely unexamined. The format of the book is unnecessarily elaborate, with the entries, many of them of trivial scientific significance, arranged in a complicated and indeed irksome sequence. Any attempt to give a complete list of species records under the various counties would be a colossal and forbidding task for any individual, and in this volume it seems ill-advised and redundant to include quite an arbitrary selection of such records. The work is undoubtedly the product of great industry and within its limits will certainly be a useful adjunct in the study of the British flora. G. TAYLOR

Founders of British Science

John Wilkins—Robert Boyle—John Ray—Christopher Wren—Robert Hooke—Isaac Newton. By J. G. Crowther. Pp. ix+296+10 plates. (London: The Cresset Press, 1960.) 35*s*. net.

IT is greatly to be hoped that no one will be misled by the title of this book: it is neither an attempt to establish an exclusively 'British science' on the model of the 'Aryan science' of the 'thirties, nor dogmatically to exclude other claimants to the title of its 'founders'. Mr. Crowther's preliminary chapter emphasizes the importance of Gilbert, Napier, Harriot, and Harvey in the 'gestatory period' of British science; indeed, as scientists, all four of these men might be held to be of greater stature than Wilkins. But 'science' for Mr. Crowther, as he has made abundantly clear in earlier writings, is no isolated activity to be followed at the beck of individual whim and genius, but is 'wholly' effective only when the course of its progress is in harmony with social needs. To illustrate this anew, he has examined with great thoroughness the contemporary sources which shed light not only on the scientific achievements of the six well-chosen 'founders' of 'organized' British science, but also on their social circumstances, aspirations, frustrations, and insight into contemporary needs In a wide-ranging survey of such a controversial question it is inevitable that the account of the facts, and even more of some of the inferences drawn from the facts, will not pass unchallenged; in pressing the argument too hard, the author is sometimes even landed in inconsistency. Also the 'close naked style', which would have pleased his hero, Wilkins, makes some of Mr. Crowther's statements appear more dogmatic than he perhaps intended. But the great virtue of his book is that, apart from its assemblage of a great deal of significant but widely scattered information, it is provocative in the best sense. It is not necessary to go the whole way with Mr. Crowther to welcome his presentation of the thesis that scientists no longer (if they ever did) pass their lives in some empyrean realm in selfless dedication to the pursuit of ultimate truth.

W. P. D. WIGHTMAN

Food Chemistry

By Prof. Lillian Hoagland Meyer. (Reinhold Organic Chemistry and Biochemistry Text-book Series.) Pp. xiv+395. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 65*s*. net.

PROF. MEYER states in her preface that the aim of her book is to provide a unified picture of foods from a chemical point of view. To this end the emphasis is on the composition of foods and the changes that occur when they are subjected to processing. The book is intended for students who have been trained in organic chemistry, but some chemical background is provided to clarify details of methods of analysis and identification. The book is descriptive rather than didactic in relation to practical work in food chemistry and is not therefore suitable as a laboratory manual. It does, however, provide a readable survey of the nature of fat, carbohydrate and protein components of common foods with additional chapters on flavour and aroma, on meat and meat products, vegetables and fruits, milk and milk products and on cereals. An appendix gives an account of food additives permitted by the most recent lists of the U.S. Food and Drug Administration. A. M. COPPING

Sampling Inspection Tables

Single and Double Sampling. By Harold F. Dodge and Harry G. Romig. Second edition, revised and expanded. (Wiley Publications in Applied Statistics.) Pp. xi+224. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 64*s*. net.

SINCE the first edition of this book appeared in 1944 it has been one of the most useful sets of tables for scientific sampling inspection. This new edition, on a much larger page, has grown almost out of recognition, mainly because of the addition of a set of diagrams of operating characteristic curves. The four chapters which precede the tables are themselves a valuable introduction to the theory and method of sampling inspection. After these one finds the curves and sampling tables for specified lot tolerance per cent defective and average outgoing quality limit, both for single and for double sampling plans. There is also (an unusual feature in tabular material) a useful index. This is an essential book for the serious student and the factory operator, written with the authority of long experience in teaching and practical application.

M. G. KENDALL

BIOCHEMISTRY OF HEREDITY: A GENERAL HYPOTHESIS

By DR. IAN LESLIE

Biochemistry Department, Queen's University, Belfast

(I) BIOCHEMICAL RELATIONSHIPS BETWEEN DEOXYRIBONUCLEIC ACID, RIBONUCLEIC ACID AND HISTONES

BECAUSE of major advances in biochemistry during the past ten years, it is now possible to think of the transfer of hereditary characteristics from parents to offspring in chemical terms. The landmarks of progress in this direction are: (1) the realization that deoxyribonucleic acid (DNA) is the primary genetic substance of all cellular organisms[1,2], and (2) the construction by Watson and Crick[3] of a molecular model of DNA, the reality of which now appears to be established[4,5]. The structure is unique, consisting of two complementary polynucleotide strands, linked together by hydrogen-bonding between the base pairs, adenine (A) to thymine (T), and guanine (G) to cytosine (C).

Other vital discoveries concern the function of ribonucleic acid (RNA), which, in contrast to DNA, is not confined to the chromosomal elements of the cells but is spread throughout the nuclear and cytoplasmic regions. The RNA molecules, like those of DNA, are now known to carry genetic information as single-stranded polynucleotides within the sequence of bases A, U (uracil), G and C. This has been demonstrated unequivocally by the discovery that the chemical alteration of the bases in RNA from tobacco mosaic virus produced a mutant virus, in which the protein had a distinctive amino-acid composition[6].

In cellular organisms it is believed the single-stranded template RNA, located in the ribosomes, directs the synthesis of the cell-specific proteins. The association between RNA and protein synthesis has been recognized for some time[7,8]; but its exact role only became known in recent years, largely through the work of Zamecnik, Hoagland and their colleagues[9-11]. Protein synthesis begins with the 'activation' of the amino-acids to produce their adenylate-enzyme complexes which then link the amino-acids to specific 'transfer' RNA molecules. This form of RNA (known as soluble or transfer RNA) is believed to direct the amino-acids into their particular sequence along the *template* RNA[10,12]. The coding operation almost certainly depends on the hydrogen-bonding of the base-pairs, A to U and G to C, but the discovery of the cipher for each amino-acid remains one of the crucial problems of current research (cf. 13, 14).

The Genetic Problem

The present article is concerned solely with the exchange of genetic information between DNA and RNA, and with the mechanism which decides whether the information will be expressed or suppressed in the differentiated cells of higher organisms.

Crick has defined the problem in straightforward and lucid terms[12]. His "central dogma" is that "the transfer of information from nucleic acid to nucleic acid, and from nucleic acid to protein may be possible, but the transfer from protein to protein or protein to nucleic acid is impossible". This is almost universally accepted, as is the concept that RNA is the messenger which carries information from the DNA of the chromosomes to the metabolic regions of the cells. In Crick's terms, the four-letter code given by the bases A, T, G and C has to be translated through RNA to form the twenty-letter amino-acid sequences, which endow proteins with their specific functions. The key to this genetic code lies, therefore, in the base composition of the messenger or *template* RNA, which, by all indications, is produced as single-stranded polynucleotides on the DNA 'master-genes'[14-16].

New information, obtained initially from cultures of human liver cells, has made it possible to formulate a general hypothesis of genetic coding and cellular differentiation. The hypothesis is presented at this stage with the minimum of elaboration. Essentially, there are three main concepts:

(1) Two genetic codes for RNA formation are operating in Nature—one in certain micro-organisms, another in the somatic cells of higher organisms.

(2) Histone proteins act as gene regulators by their ability to 'stabilize' one polynucleotide strand and depolymerize or 'disjoint' the complementary strand which is derived from the 'soluble' or 'transfer' RNA.

(3) In the differentiated cells of higher organisms, part of the histone allows the RNA templates to act as protein synthesizers; another form of histone, located in the nuclei, has the function of suppressing the template function of the associated RNA.

The Base Composition of Template RNA

Human liver cells (strain *HLM*) have been cultured in sufficient amounts for their fractionation into nuclei, combined particles (nuclei, mitochondria, large microsomes), and ribosomes. The nuclei were cleaned by treatment with 0·25 M sucrose, 0 01 M citric acid at pH 3, and the combined particles were spun down at 18,000g for 30 min. from 0·44 M sucrose buffered at pH 7·4. The ribosomes were obtained by treatment of the microsomal pellets with surface-active agents in sucrose medium and sedimentation at 105,000g for 2 hr. The determination of base ratios in RNA and DNA gave the following information, which will be published in greater detail elsewhere[20].

(1) The mean DNA base composition, A : T : G : C is 30 : 30 : 20 : 20 and close to the values previously found for human cells[21].

(2) The total RNA of the combined particles has base ratios for A : U : G : C equal to 19 : 18 : 31 : 32.

(a) Duplex DNA	Per 100 bases	(b) Duplex RNA	Per 100 bases
-A—T-	30 A	-A—U-	19 A
-T—A-	30 T	-U—A-	18 U
-G—C-	20 G	-G—C-	31 G
-C—G-	20 C	-C—G-	32 C
	(determined)		(determined)

(c) RNA isolated from nuclear fraction*

RNA template	RNA second strand	Hence duplex RNA	Per 200 bases	Per 100 bases
24 -A	24 -U	24 -A—U- 24	39 A	19·5 A
15 -U	15 -A	15 -U—A- 15	39 U	19·5 U
37 -G	37 -C	37 -G—C- 37	61 G	30·5 G
24 -C	24 -G	24 -C—G- 24	61 C	30·5 C
(determined)	(deduced)			

* Substitution in the 'RNA template' column of the ratios A : U : G : C = 14 : 25 : 48 : 13, obtained for the RNA of acid-extracted combined particles, gives the same values in the last column.

Fig. 1. The base compositions of duplex forms of DNA and RNA and their relationship to single-strand RNA isolated from human liver cells (*HLM* strain)

These may reasonably be called the inverse of the DNA ratios ; ideally, they would be 20 : 20 : 30 : 30. For convenience, such base compositions in which A = T or U and G = C will be called 'symmetrical'.

(3) Extraction of the combined particles with ice-cold dilute sulphuric acid removed histone protein and about 50 per cent of the original RNA. The composition of the residual RNA was found to be radically changed from the symmetrical pattern of total RNA. The ratios A : U : G : C of 14 : 25 : 48 : 13 are surprisingly close to those of post-microsomal RNA's isolated from guinea-pig pancreas[22].

(4) For the total RNA of citric acid-washed nuclei the base ratios are 24 : 15 : 37 : 24 and are almost identical to values for RNA isolated from rat-liver ribosomes and purified by column chromatography on 'Ecteola' cellulose[23]. Ribosomal RNA from *HLM* cells also has an 'asymmetrical' composition (19 : 18 : 36 : 27).

(5) Acid extraction of histone protein from nuclear and ribosomal fractions caused no loss of RNA and scarcely any change in the asymmetrical base ratios.

The interpretation of these results is given diagrammatically in Fig. 1. The symmetrical ratios for DNA are a recognized feature of its duplex structure (Fig. 1*a*). It is proposed that the symmetrical ratios for total RNA in the combined particles are a consequence of its duplex form in these preparations. The asymmetrical RNA's, on the other hand, are thought to be different molecular populations of *single-stranded template* RNA. If this proposition is correct, the duplex base ratios derived from them arithmetically should be the same as those found for total RNA in combined particles. These are calculated for nuclear RNA (Fig. 1*c*), on the assumption that A bonds to U and G to C. The agreement between the deduced values and symmetrical RNA (Fig. 1*b*) is, in fact, found to be very close.

With the knowledge that the duplex and single-stranded forms of RNA can be isolated by appropriate procedures, and can be recognized by the symmetry or the particular asymmetry of the base compositions, it has been possible to detect these two forms of RNA in the results of other investigators. In some instances, the conclusion that the RNA has been isolated as single-stranded polynucleotide is associated with metabolic evidence suggesting its template function[22,24,25].

Transfer of Template RNA from Nucleus to Cytoplasm

In the present work with human cell cultures, it has been possible to show that the asymmetrical or template RNA is produced in the nucleus and moves to the cytoplasm during interphase. This transfer is a vital feature of present concepts of genetic coding and has been studied intensively in recent years (cf. ref. 26). The movement of RNA from nucleus to cytoplasm has already been made visible in human cells by labelling the nucleic acid with tritiated-cytidine and applying the autoradiographic technique to the coverslip cultures removed at intervals over the subsequent twenty hours[27]. However, in these circumstances, the labelled polynucleotide could consist of both the template and 'transfer' parts of RNA. This doubt has been eliminated by labelling the nucleotides with phosphorus-32 and isolating them from the asymmetrical RNA's prepared from the nuclear and ribosomal fractions.

The discovery that 5-fluoro-2-deoxyuridine inhibits DNA synthesis without immediate effect on RNA formation makes it possible to block mitosis and keep the majority of the cells in the interphase state[28]. Preliminary tests showed that RNA and protein in the *HLM* cell cultures would increase by about 50 per cent in 24 hr., without any concurrent increase in DNA formation. With this experimental system, the changing pattern of labelling in the RNA nucleotides was followed in the nuclear and ribosomal fractions at different times after the cells had been in contact with isotopic medium for one hour. The isotopic medium was completely removed, and the cells continued to metabolize in the 'cold' medium, which was replaced again one hour later to reduce still further the metabolic pool of radioactive phosphate.

The first obvious feature of the labelling was that the specific activities of the RNA nucleotides from the nuclei were four times those of ribosomes at one hour and they were still twice as high at ten hours. After 24 hr. the levels of radioactivity were the same in all fractions. This rapid labelling of nuclear RNA, followed by a gradual levelling-out of the difference between nuclear and ribosomal RNA, is a well-established sequence of events in animal cells[29]. However, unless the labelled nucleotides are known to come from asymmetrical template RNA, it is not possible to conclude from the prior labelling in the nuclei that it eventually moves to the cytoplasm.

When it was realized that RNA had been isolated in template or near-template form (as in ribosomes), the change in pattern of the labelling of the nucleotides with phosphorus-32 with time became understandable. The results of two tests are shown in Table 1. As the specific activities are all expressed relative to the value of 100 for adenine, the more rapid labelling of the nuclear RNA is concealed. The values are intended to emphasize important differences between the related nucleotides, adenylic acid and guanylic acid on one hand, and between uridylic acid and cytidylic acid on the other. It is seen that specific activities are always higher for adenylic acid and uridylic acid than for guanylic acid and cytidylic acid, and that the ratios, adenylic : guanylic and uridylic : cytidylic are greatest in ribosomal RNA at the short labelling-time of one hour. By comparison, the corresponding ratios for nuclear RNA are nearer unity or 'less asymmetrical' at each time of sampling.

The interpretation of these results rests on the knowledge that, in the synthesis of purine nucleotides,

Table 1. EVIDENCE FOR THE MOVEMENT OF TEMPLATE RNA FROM THE NUCLEUS TO CYTOPLASM DURING INTERPHASE IN CULTURES OF *HLM* CELLS

Source of RNA	Duration of phosphorus-32 metabolism* (hr.)	Relative specific activities of nucleotides (Adenylic acid = 100 counts/min./10 μgm.P)			
		Adenylic acid	Uridylic acid	Guanylic acid	Cytidylic acid
Nuclei	1	100	108	84	85
Ribosomes	1	100	130	49	51
Nuclei	6	100	119	105	108
Ribosomes	6	100	99	66	75
Nuclei	10	100	106	97	94
Ribosomes	10	100	114	87	86
Ribosomes	23	100	109	98	97

* The culture medium contained 0·1 μgm. 5-fluoro-2-deoxyuridine/ml. from 4 hr. before inclusion of 5 μc. $^{32}PO_4$/ml. for a period of 1 hr. Isotopic medium was replaced by non-isotopic medium at 1 and again at 2 hr., and the cells were harvested at times indicated.

adenylic acid precedes in time guanylic acid, and in the synthesis of pyrimidine nucleotides, uridylic acid precedes cytidylic acid[29–31]. This should lead to an initial unevenness of nucleotide labelling which can be expected to disappear when equilibrium with radioactive inorganic phosphate is achieved. (This is found even when the RNA is degraded to 2′(3′)-nucleotides, since in asymmetric RNA there is at least 60 per cent of guanine and cytosine.)

The only likely explanation which can reconcile the coincidence of faster labelling of nuclear RNA with greater asymmetry of radioactivity within ribosomal RNA is that template RNA is formed within the nucleus and moves out to the cytoplasm. The gradual progression towards uniform labelling of nucleotides in both fractions as time proceeds is fully consistent with this interpretation.

Ribonuclease Activity of Histone Protein

The most remarkable feature about histone protein has been the lack of information about its metabolic or enzymic activity. (This was justifiably said of the nucleic acids less than ten years ago (cf. ref. 12).) It has long been recognized as the companion-protein of DNA in animal and plant cells, and the view is still held that histone, like DNA, is confined to the cell nucleus. The one role which has been consistently claimed for it is that the β-histone from nuclei is cell-specific and acts as a gene-inhibitor[32,33]. This has remained hypothetical, because the concept depended on observed differences in composition and electrophoretic behaviour of the proteins rather than on the demonstration of their mode of action.

Within the past two years, the presence of histone-like protein has been confirmed in the ribosomes prepared from animal and plant cells[34–36], and is reported in the smallest ribosomes from *Escherichia coli*[37]. When it became apparent that histone might not be confined to the nucleus, an investigation was begun on its distribution between the nuclei and ribosomes of *HLM* cells. The protein was extracted with ice-cold 0·5 *N* sulphuric acid and precipitated with ethanol[38], before being purified by column chromatography on carboxymethylcellulose (Whatman)[39]. This work was later extended to the histones of guinea pig liver, and the new information which it yielded is now briefly summarized.

(1) The amino-acid composition of the acid-extracted protein from ribosomes of *HLM* cells was shown by paper chromatography to be similar to that of histone[40]. The average arginine concentration was 16 per cent.

(2) The nuclear histones from both *HLM* cells and liver produced four fractions in the eluent from the column. Fraction I was largely polynucleotide material; II, III and IV were histones. Fraction III was arginine-rich (average 22 per cent) and it probably corresponds to the β-histone of Cruft, Mauritzen and Stedman[33].

(3) By contrast, the ribosomal extracts from both *HLM* cells and liver produced only three peaks, corresponding to I, II and IV above. It can be concluded that only histones II and IV are associated with the template RNA which moves from the nucleus to cytoplasm. The profiles of ribosomal and nuclear histones isolated from *HLM* cells are shown in Fig. 2.

There were a number of reasons for proceeding to test the eluted histones for their ribonuclease activities. Chief among them were the reports that highly purified ribosomes contained latent forms of this enzyme, which became active only on releasing the protein from its associated RNA[41,42]. It was also observed that the profiles for the ribosomal histones were similar to those for ribonuclease released from pancreas microsomes by dilute sulphuric acid and separated by column chromatography[43]. The following results were obtained with histones from *HLM* cells and guinea pig liver[44].

(4) Each histone peak from the nuclear and ribosomal preparations showed ribonuclease activity towards yeast RNA[45]. The activities of nuclear histones were higher at *p*H 7·8 (alkaline histone-ribonuclease) than at *p*H 5 (acid histone-ribonuclease), in general agreement with earlier observations on the total enzyme activity in liver nuclei[46]. With the ribosomal histones from *HLM* cells, depolymerization occurred at *p*H 7·8, but the same protein showed virtually no activity at *p*H 5; Tashiro[42] and Roth[47] both report that ribosomes contain latent alkaline

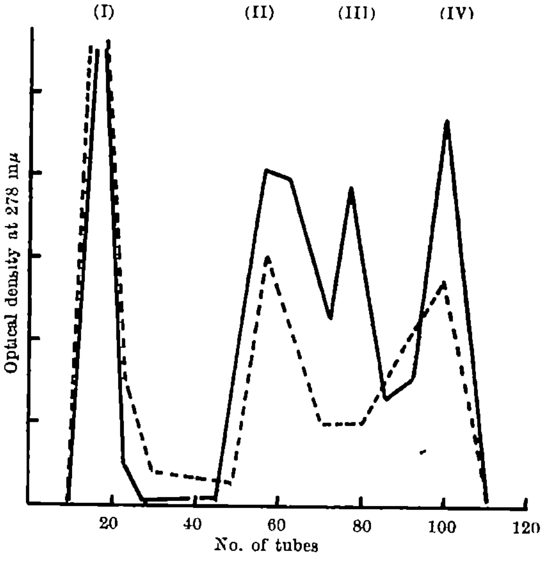

Fig. 2. Histones of human liver cells (*HLM* cells) fractionated by gradient elution from 10-cm. columns of carboxymethylcellulose (Whatman) —, nuclei; – – –, ribosomes

but not acid ribonuclease. The distribution within these cells of the histone-nucleases with these *p*H optima has still to be fully delineated.

(5) Ribonucleic acid, prepared from guinea pig liver, showed no depolymerization at *p*H 5 in the presence of excess of the combined nuclear histones from this organ. (Excess of histone is present in the assay when there is more than one histone amino-acid for every nucleotide in the substrate RNA.) The same RNA preparation, which had been purified by removal of nucleotide material soluble in ice-cold *N* perchloric acid in 1 hr., was depolymerized by bovine pancreatic ribonuclease and by *HLM* nuclear histone under identical conditions. Confirmation of enzymic activity of the nuclear histone was obtained concurrently with yeast RNA, likewise purified by mild perchloric acid treatment.

(6) The above observations on histone-nuclease activity are found to depend on the presence of sodium chloride of between 0·05 and 0·3 *M* in the assay medium. Further work on the effects of sodium, potassium and magnesium ions is necessary for a proper understanding of histone-nuclease action.

(7) As regards the nature of the specially prepared RNA used in these tests, there is sufficient evidence, based upon the recognition of the base compositions of single-strand and double-helix RNA, to indicate that it is the single-strand, template form of the nucleic acid.

This work on the interaction between histone fractions and RNA from different sources is continuing, but it already shows that histones can act as depolymerases towards RNA, if the RNA is not their associated template form. Their stabilizing action towards template RNA is indicated by the absence of any breakdown when homologous RNA was incubated with excess of the combined nuclear histone in the absence of saline and magnesium ions. This suggests that RNA is stabilized by histone when the ionic conditions promote bonding between the RNA and histone moieties.

Genetic Function of Histone-nuclease

It is proposed that each RNA template has specific stabilizing histones, which can act as depolymerases towards polyribonucleotides of different base composition. Experimental support for this concept is implicit in the present results and in three illuminating papers on the latent ribonuclease activity in ribonucleoproteins[42,47,48]. There are also such clear-cut distinctions between the effects on 'synthetic' polyribonucleotides as the ability of bovine pancreatic ribonuclease and an alkaline ribonuclease from rat liver to degrade polyuridylic acid but not polyadenylic acid[49]. The more subtle aspects of the inverse behaviour of different nucleases have been described by Hilmoe[50].

Logically, it is a short step from this view of histone-ribonuclease action to the concept that each stabilizing histone of single-strand template RNA is the depolymerase for its complementary strand formed during protein synthesis. Although a reasoned case could be made for this particular 'all-or-none' action of ribonuclease, it would be premature to do so until it is known whether the minor nucleotides (pseudouridine, 5-methylcytosine, 6-methylaminopurine, etc.), found mainly in soluble RNA[51], are also part of the complementary strand.

It is postulated that histones, like template RNA, are formed on the DNA genomes and may have the function of stabilizing the RNA templates while acting as depolymerases towards the complementary strands. If separated from the RNA, they will depolymerize any unprotected RNA molecules of different base composition. It follows that the association of nucleic acid with specific histone-nucleases[48] may be the means of suppressing the continuity of the polynucleotide strands which would pass on the 'wrong' information in protein synthesis.

Stent[52] has discussed this problem of 'active' and 'passive' strands in relation to genetic coding systems, and from Hotchkiss[53] has come the suggestion that "theories of DNA replication should take into account the enormous stability that one single-helix running in one direction could have towards an enzyme actively engaged in exchanging base residues at the end of a chain which is being synthesized in close proximity along an opposite direction". These concepts form an integral part of the hypothesis that there are in existence two coding systems for exchanging genetic information between DNA and RNA.

(2) GENETIC CODING SYSTEMS IN CELLS AND VIRUSES

Four coding systems have been proposed for the transfer of genetic information from DNA genomes to the RNA templates required for protein synthesis[52,54–56]. All involve the assumption that the purines and pyrimidines *or* their base-pairs, A–T and G–C, along the DNA chains determine the base sequence in the derived single-strand RNA. The ciphers for these codes are shown in Table 2. Three envisage the formation of RNA on duplex DNA and the fourth adopts RNA base-pairing rules, analogous to those in DNA, for producing an RNA strand from a single gene-strand. This last code is attributed to Rich[55], as he has presented the case for it in some detail.

Zubay[54] and Sinsheimer[56] developed their coding systems from the observation of certain trends in the base compositions of RNA and DNA in metazoan and unicellular organisms. The basis of Zubay's code is the near-equivalence in animal cells of the ratio $(G + C)/(A + U)$ in nuclear RNA with the ratio $(A + T)/(G + C)$ in the associated DNA. This led him to propose that the base-pairs A—T and T—A locate either cytosine or guanine on single-strand RNA, according to their orientation along the double-helix; similarly, G—C and C—G locate the position of the bases adenine and uridine, and thus determine the sequence along the RNA strand. The four possible arrangements of this coding system are given in Table 2.

Sinsheimer's coding system was designed to reconcile the paradox of the apparent co-existence of DNA of widely different composition with RNA of more uniform composition in a range of bacterial organisms. Essentially, his system allows the base-pair C—G to be substituted for A—T without altering the base sequence of the derived RNA. Again there are four possible combinations of this code, as shown in Table 2.

The individuality of the coding systems of Rich and Stent[52] lies in the manner of their operation. Stent envisages the growth of RNA as a third strand in the deep groove of the DNA duplex, while Rich

Table 2. POSSIBLE CODING SCHEMES FOR THE TRANSFER OF INFORMATION BETWEEN DNA AND RNA

Coding system	Proposed rules*	Possible combinations for transfer. DNA to RNA. RNA bases: Adenine	Uracil	Guanine	Cytosine
Duplex: DNA to single-strand RNA	*Zubay* (ref. 54) *AT* or *TA* gives G or C *GC* or *CG* gives A or T	(*a*) GC	CG	AT	TA
		(*b*) CG	GC	TA	AT
		(*c*) GC	CG	TA	AT
		(*d*) CG	GC	AT	TA
	Stent (ref. 52) As in next column	TA	AT	CG	GC
	Sinsheimer (ref. 56) *AT*/*CG* give G/U or A/C *TA*/*GC* give C/A or U/G	(*a*) TA	CG	AT	GC
		(*b*) AT	GC	TA	CG
		(*c*) CG	TA	GC	AT
		(*d*) GC	AT	CG	TA
Single-strand DNA to single-strand RNA	cf. Rich (ref. 55) based on Watson-Crick base-pairing	T	A	C	G

* Italics indicates DNA base-pairs.

suggests, for stereochemical reasons, that a single strand of RNA could form on a strand of DNA, producing a transient hybrid molecule. Both these codes have a single cipher for the four RNA bases by which, for example, A and A—T locate the position of uridine in the RNA strand. They adhere, in effect, to the hydrogen-bonding rules for the DNA duplex.

A vital difference underlies this similarity in their 'message'. In the single-strand system of Rich, each strand from duplex DNA should be capable of producing an RNA template. Owing to their opposite polarity, these strands would carry contradictory information to the sites of protein synthesis. Either the concept of 'active' and 'passive' DNA strands must be invoked[52], or a mechanism must exist for suppressing one template while eliciting the expression of the other.

Conditions for Testing Genetic Coding Systems

Knowing the base compositions of single-strand RNA and the conditions for isolating it in this form, it is possible to test the validity of these four coding systems. Thoroughly rigorous criteria for such tests would require:

(1) Independent confirmation that the template RNA was single-stranded by physicochemical studies or by the demonstration of its ability, when in ribosomes, to direct specific protein synthesis.

(2) The isolation from the same tissue of RNA in its double-helical form, and the knowledge that its base composition agrees with the duplex ratios calculated from the single-strand or asymmetric RNA as shown in Fig. 1.

(3) An established base composition for the DNA of the species.

For the present, it is not possible to satisfy fully these conditions. Since the hypothesis is intended to lead to experimentation and discussion, it will be developed from the available information.

Table 3 gives the base compositions of RNA and DNA from human cells, rat liver and pea seed roots. The reasons for the assumption that two RNA complexes have been isolated from the human liver cells in their single-strand form are given above. The extent of the base-ratio asymmetry can be judged from the factor in the second-last column of Table 3. This increases as the numerical gaps between adenine and uridine, and between guanine and cytosine, widen. The values of the factors are, of course, very low or zero in duplex forms of RNA and DNA.

The base ratios for single-strand RNA of rat liver are those of RNA II isolated by column chromatography on 'Ecteola' cellulose by Goldthwait[23], who observed that it was similar to post-microsomal RNA and did not act as 'transfer' RNA, unlike the accompanying RNA. It is seen that the duplex composition, calculated from the base ratios of RNA II, resembles closely a determined symmetrical RNA, isolated from whole-cell preparations by Elson and Chargaff[57]. The inverse patterns of the base compositions of duplex forms of DNA and RNA are again evident in these cells, and this relationship is, in fact, a characteristic feature of the nucleic acids in animal and plant cells.

The asymmetrical RNA of pea seed roots came from the nucleic acid, which remained in the thin transverse segments of the tissue after extraction with *N* perchloric acid at 2–4° over 30 hr.[58]. There is evidence that the degradation of the complementary strand occurs under such conditions[30] and the increased amounts of residual RNA found in the more mature cells[58] are consistent with its template nature.

Genetic Coding in Animal and Plant Cells

The consequences of applying the coding systems of Zubay[54] and Rich[55] to the data for human liver cells are illustrated in Fig. 3. By using the ciphers given in Table 2, the composition of the DNA necessary to operate can be calculated from the asymmetric base-ratios of RNA. Only the first arrangement of the Zubay code is applied, since the others obviously give

Table 3. BASE COMPOSITIONS AND ASYMMETRY FACTORS FOR SINGLE-STRAND AND DUPLEX FORMS OF RNA AND DNA FROM METAZOAN CELLS

Species and tissue	Nucleic acid	Base composition (number per 100 bases): Adenine	Uracil/thymine[a]	Guanine	Cytosine	Asymmetry factor[b]	Ref. No.
Human liver (*HLM* cells)	Single-strand RNA[c]	24	15	37	24	22	—
	[d]	14	25	48	13	46	—
	Duplex RNA[e]	19	18	32	31	2	—
	Duplex DNA	30	30	20	20	0	—
Rat liver	Single-strand RNA	25	13	38	24	26	23
	Duplex RNA-calc.[f]	19	19	31	31	0	—
	Duplex RNA-deter'd.	18	20	32	30	4	57
	Duplex DNA	29	29	21	21	0	21
Pea seed roots[g]	Single-strand RNA	27	17	39	17	22	58
	Duplex RNA-calc.[f]	22	22	28	28	0	—
	Duplex DNA	29	30	20	21	2	58

[a] Thymine replaces uracil in DNA.

[b] This factor is the sum of the differences between A and U and G and C.

[c] Is isolated from nuclear fraction washed with sucrose-citric acid medium.

[d] Is isolated from combined-particles after histone extraction (see Fig. 1 and text).

[e] Is isolated directly from combined-particles.

[f] Calculated from single-strand RNA as shown in Fig. 1c.

[g] Residual RNA and DNA in tissue after treatment with ice-cold *N* perchloric acid for 30 hr.

(*a*) Base-pair coding (for example, Zubay[44]).

RNA nuclear	DNA base-pairs	Hence in DNA	Per 100 bases	Conclusion
24 –A	G—C (24)	A = 37 + 24	30·5 A	Similar to determined composition of DNA. *Code valid*
15 –U	C—G (15)	T = 24 + 37	30·5 T	
37 –G	A—T (37)	G = 24 + 15	19·5 G	
24 –C	T—A (24)	C = 15 + 24	19·5 C	

(*b*) Single-strand coding (cf. Rich[44]).

RNA nuclear	DNA template	Hence DNA second strand	DNA bases	Per 100 bases	Conclusion
24 –A	T– 24	A– 24	A = 24 + 15	19·5 A	Reverse of determined composition of DNA. *Not valid*
15 –U	A– 15	T– 15	T = 15 + 24	19·5 T	
37 –G	C– 37	G– 37	G = 37 + 24	30 5 G	
24 –C	G– 24	C– 24	C = 24 + 37	30 5 C	

N.B. The same results are obtained with the base composition of RNA isolated from combined-particles after histone extraction

Fig. 3. Procedures for testing coding schemes illustrated with data obtained from human liver cells (*HLM*)

the same result. If the derived composition agrees with the determined one for the RNA, the code may be said to be valid. If it gives the inverse pattern to the actual one, the code is clearly not valid. This unequivocal answer is obtained when the double-strand system of Zubay is compared with the single-strand code of Rich. The DNA base ratios in Fig. 3*a* are very close to the observed values, while in Fig. 3*b* they are clearly the inverse.

As Table 4 shows, such a straightforward distinction cannot always be made when testing the validity of the codes. While there is no doubt that single-strand coding does not apply to animal and plant cells, ambiguity of decision appears when the first two arrangements of the Sinsheimer code are compared with the Zubay system. An arbitrary rating system has been devised to assist in the judgment of their validity (see footnote to Table 4). It indicates a slightly greater identity between derived and determined values for DNA when the Zubay code is applied, since for the rat and pea data the system is *probably* valid, whereas with the Sinsheimer code it is *possibly* valid. However, it cannot be concluded on such grounds that one operates and the other does not. More cogent reasons for rejecting the Sinsheimer code in favour of the Zubay system will be given later.

The vital feature of these tests is that they indicate that the exchange of genetic information between single-strands of DNA and RNA does not occur in these metazoan cells, and that the coding system which operates involves the formation of template single-strand RNA by the genetic activity of the duplex form of DNA.

Genetic Coding in Unicellular Organisms

The DNA and RNA of unicellular organisms have similar base-ratios for their symmetrical forms (Table 5). This is in complete contrast to the inversions characteristic of these nucleic acids in animal and plant cells (cf. Table 3). Where DNA from *Tetrahymena*[59] has adenine plus thymine in excess of guanine plus cytosine, the symmetrical RNA from this protozoan is similarly weighted. Likewise, when guanine and cytosine are high, as in the DNA of *Aerobacter aerogenes*[60], *Escherichia coli*[60] and *Mycobacterium tuberculosis*[61], these bases predominate in the duplex RNA form.

There are sound reasons for regarding base-ratios in Table 5 which have asymmetry factors of 12 and 13 as single-stranded forms of the nucleic acid. The RNA of *Aerobacter aerogenes* was prepared by a series of fractionations in the presence of surface-active agents[62]. All the recent information on the ribosomes from *Escherichia coli* suggests that they contain active template RNA[25,63,64]. In the analysis of the base-ratios of RNA from the 30S, 50S and 70S ribosomes, Spahr and Tissières[65] appear to have isolated the nucleic acid in its template form.

When the different coding systems are tested, as in Table 6, with these base-ratios, it becomes obvious that the method of exchanging genetic information between DNA and RNA in some micro-organisms is entirely different from that operating in animal and plant cells. The systems of Zubay and Sinsheimer (codes *a* and *b*, Table 2) are unmistakably eliminated by the data for unicellular organisms. Instead, the bacterial cells could employ either the duplex systems of Stent and Sinsheimer (codes *c* and *d*, Table 2) or the single-strand information exchange of Rich. The duplex forms of DNA and RNA in *Mycobacterium*

Table 4. APPLICATION OF CODING SCHEMES TO DATA FOR RNA AND DNA BASE COMPOSITIONS OF METAZOAN CELLS (TABLE 3)

Species and coding system*	Nucleic acid	Base composition (number per 100 bases)				Validity of code‡
		Adenine	Uracil/thymine†	Guanine	Cytosine	
Man	*Determined*					
	Single-strand RNA§	24	15	37	24	
	Duplex DNA	30	30	20	20	
	Derived					
Zubay (*a–d*)	Duplex DNA	30 5	30 5	19 5	19·5	Valid
Stent (1 code)		19 5	19 5	30 5	30·5	Not valid
Sinsheimer (*a–b*)		30 5	30 5	19 5	19·5	Valid
Sinsheimer (*c–d*)		19 5	19 5	30 5	30·5	Not valid
Rich (1 code)		19 5	19 5	30 5	30 5	Not valid
Rat	*Determined*					
	Single-strand RNA	25	18	33	24	
	Duplex DNA	29	29	21	21.	
	Derived					
Zubay (*a–d*)	Duplex DNA	31·0	31 0	19 0	19 0	Probable
Stent (1 code)		19 0	19·0	31 0	31·0	Not valid
Sinsheimer (*a–b*)		31 5	31 5	18·5	18 5	Possible
Sinsheimer (*c–d*)		18·5	18 5	31·5	31 5	Not valid
Rich (1 code)		19 0	19 0	31 0	31 0	Not valid
Pea	*Determined*					
	Single-strand RNA	27	17	39	17	
	Duplex DNA	29	30	20	21	
	Derived					
Zubay (*a–d*)	Duplex DNA	28 0	28 0	22 0	22 0	Probable
Stent (1 code)		22 0	22 0	28 0	28 0	Not valid
Sinsheimer (*a–b*)		33·0	33 0	17 0	17 0	Possible
Sinsheimer (*c–d*)		17·0	17 0	33 0	33 0	Not valid
Rich (1 code)		22·0	22 0	28 0	28 0	Not valid

* Ciphers for the four codes are shown in Table 2.

† Thymine replaces uracil in DNA.

‡ Rating system—*valid* if within ±1 of base number in determined DNA; *probable* if ±2 of base number; *possible* if within ±4 of base number; *not valid* if the inverse of determined DNA.

§ Application of the base composition obtained by method *d* (Table 3) leads to the same conclusions.

Table 5. BASE COMPOSITIONS AND ASYMMETRY FACTORS FOR SINGLE-STRAND AND DUPLEX FORMS OF RNA AND DNA FROM UNICELLULAR ORGANISMS

Species	Nucleic acid	Base composition (number per 100 bases)				Asymmetry factor^b	Ref. No.
		Adenine	Uracil/thymine^a	Guanine	Cytosine		
Aerobacter aerogenes	Single-strand RNA	26	20	30	24	12	62
	Duplex RNA-calc.^{c}	23	23	27	27	0	—
	Duplex DNA	21	21	29	29	0	60
Escherichia coli	Single-strand RNA	24	21	32	23	13	65
	Duplex RNA-calc.^{c}	22	22	28	28	0	—
	Duplex DNA	21	21	29	29	0	60
Mycobacterium tuberculosis^d	Duplex RNA	20	19	31	29	3	61
	Duplex DNA	18	17	33	32	2	61
Tetrahymena^d	Duplex RNA	31	30	20	19	2	59
	Duplex DNA	37	37	11	15	4	59

^{a} and ^{b} See footnotes in Table 8.
^{c} Calculated from RNA (single-strand) by procedure shown in Fig. 1c.
^{d} No values for RNA (single-strand) available.

tuberculosis[61] and *Tetrahymena*[59] show that they probably adopt a similar coding system.

It is found that the compositions of DNA and RNA from a wide range of bacteria confirm in a general way these conclusions. Belozersky and Spirin[60] noted that extremes in DNA composition (cf. Table 5) were accompanied by RNA of more uniform composition. This becomes understandable now that it is apparent that RNA must be isolated in either its single-strand or duplex form in order to reveal its correct base relationship to DNA. The correlation was, however, sufficiently good for the authors to suggest that "part at least of the ribonucleic acid depends on the deoxyribonucleic acid, and that the two nucleic acids may even be similar with respect to their base compositions". Perhaps it can now be said that they are—in their duplex forms.

Nature of Viral Nucleic Acid

Single-strand nucleic acids are known to form the core of two viruses. They are the RNA of tobacco mosaic virus[66,67] and the DNA of the small bacteriophage Φ*X*174, investigated by Sinsheimer[68]. Their asymmetrical base-ratios[68,69] are shown in Table 7 along with the compositions of other viruses and of the RNA or DNA of the host cells. Influenza[70] and poliomyelitis[71] viral RNA's are seen to have similar base asymmetry to 'single-strand' RNA of the chick chorioallantoic cells[72] and *HLM* cells.

A fascinating relationship between the nucleic acids of virus and host cell was revealed when the duplex base-ratios were calculated from single-strand values. With animal, plant and bacterial viruses there was, in each case, a 'cross-over' in the pattern of bases in viral nucleic acid and host RNA or DNA (cf. Table 7). The precision of this inversion is particularly impressive with the viruses of tobacco leaf and *Escherichia coli*. (The similar relationship between polio virus Type I and influenza *LEE* (*B*) strain and host RNA is based on the assumption that these viruses also contain single-strand RNA.)

It may be concluded that, for all the above viruses, the RNA or the DNA represents the 'antithesis' in base composition of the template nucleic acid of the host cells. The 'cross-over' pattern also exists between the duplex DNA in certain viruses (for example, *T*2*r* and *T*7)[73] and the nucleic acids of the infected *Escherichia coli* as seen in Table 7. There is one puzzling exception, quite apart from the problems arising from these observations; the duplex base ratios of bacteriophage *T*3 are unique in having the same base-pattern as the DNA of *Escherichia coli*. It is possible, however, that this aberration can be reconciled, since fraction IV of the bacterial DNA, isolated by column chromatography[74], has a base-pattern which is the inverse of the viral DNA and of the total DNA of the cells (cf. Table 7).

Possible Interpretations

No attempt will be made in this article to explore the genetic consequences of these findings. They cover a wide range of knowledge, and time is needed for reflexion and discussion. For the present, it would seem that the relationships found to exist between DNA, RNA and the histones are more than fortuitous, and they suggest to me a number of concepts which may help to make genetic processes understandable in biochemical terms.

(1) The exchange of genetic information between DNA and RNA is carried out by one coding system in animal and plant somatic cells and by another in some unicellular organisms.

(2) Such ambivalence of code might be provided by the Sinsheimer system, since two of its ciphers could operate in metazoan cells (Table 4) while the other two could operate in micro-organisms (Table 6). It is, therefore, not 'indeterminate', as Sinsheimer intended it to be[68]. There are other conceptual reasons which make its 'universality' improbable. For example, it seems too insignificant a change in

Table 6. APPLICATION OF CODING SYSTEMS TO DATA FOR RNA AND DNA BASE COMPOSITIONS OF UNICELLULAR ORGANISMS (TABLE 5)

Species and coding systems*	Nucleic acid	Base composition (number per 100 bases)				Validity of code‡
		Adenine	Uracil/thymine†	Guanine	Cytosine	
Aerobacter aerogenes	*Determined*					
	Single-strand RNA	26	20	30	24	
	Duplex DNA	21	21	29	29	
	Derived					
Zubay (*a*–*d*)	Duplex DNA	27·0	27·0	23·0	23·0	Not valid
Stent (1 code)		23·0	23·0	27·0	27·0	Possible
Sinsheimer (*a*–*b*)		28·0	28·0	22·0	22·0	Not valid
(*c*–*d*)		22·0	22·0	28·0	28·0	Probable
Rich (1 code)		23·0	23·0	27·0	27·0	Possible
Escherichia coli	*Determined*					
	Single-strand RNA	24	21	32	23	
	Duplex DNA	24	24	26	26	
	Derived					
Zubay (*a*–*d*)	Duplex DNA	27·5	27·5	22·5	22·5	Not valid
Stent (1 code)		22·5	22·5	27·5	27·5	Probable
Sinsheimer (*a*–*b*)		28·0	28·0	22·0	22·0	Not valid
(*c*–*d*)		22·0	22·0	28·0	28·0	Possible
Rich (1 code)		22·5	22·5	27·5	27·5	Probable

*, † and ‡ as in Table 4.

Table 7. BASE COMPOSITIONS AND ASYMMETRY FACTORS FOR SINGLE-STRAND AND DUPLEX FORMS OF RNA AND DNA FROM VIRUSES AND THEIR HOST CELLS

Virus and host cells	Nucleic acid	Base composition (number per 100 bases)				Asymmetry factor*	Ref. No.
		Adenine	Uracil/thymine	Guanine	Cytosine		
Poliovirus Type I	Single-strand RNA	31	25	26	18	14	71
	Duplex RNA-calc.	28·0	28·0	22·0	22·0	0	—
HLM cells (human)	Single-strand RNA	24	15	37	24	22	—
	Duplex RNA-calc.	19·5	19·5	30·5	30·5	0	—
Influenza *LEE(B)* strain	Single-strand RNA	23	36	18	23	18	70
	Duplex RNA-calc.	29·5	29·5	20·5	20·5	0	—
Chorio-allantoic membrane (chick)	Single-strand RNA	22	18	33	27	10	72
	Duplex RNA calc.	20·0	20·0	30·0	30·0	0	—
Tobacco mosaic virus	Single-strand RNA	29	28	25	18	8	69
	Duplex RNA-calc.	28·5	28·5	21·5	21·5	0	—
Tobacco leaf	Single-strand RNA	24	19	33	24	14	69
	Duplex RNA-calc.	21·5	21·5	28·5	28·5	0	—
Bacteriophage $\Phi X174$	Single-strand DNA	24	31	25	20	12	68
	Duplex DNA-calc.	27·5	27·5	22·5	22·5	0	—
Escherichia coli	Single-strand RNA	24	21	32	23	12	65
	Duplex RNA-calc.	22·5	22·5	27·5	27·5	0	—
Bacteriophages *T2r*	Duplex DNA	32	32	18	18†	0	73
T3	Duplex DNA	24	24	26	26	0	73
T7	Duplex DNA	26	26	24	24	0	73
Escherichia coli	Duplex DNA	24	24	26	26	0	60
	Duplex DNA (IV)‡	27	27	23	23	0	74

* As defined in Table 3. † Includes 5-methylcytosine. ‡ See text (ref. 74).

coding to account for the evolution from asexual life to the multicellular and sexual nature of higher organisms.

(3) Zubay devised his coding system to account for inversion of the base ratios for DNA and RNA in animal nuclei. The inversion is confirmed for their symmetrical forms, and it is seen that four stereochemical arrangements of the Zubay system could function in animal and plant cells (Table 4). Zubay[75] has himself provided reasons for rejecting the code, but its observed validity in some metazoan cells leads one to suggest that mating genomes or paired chromatids[76] produce single-strand template RNA within a three-dimensional nucleohistone complex[77,78].

(4) The systems of Stent[52] and Rich[54] are valid for micro-organisms, and the Zubay code is definitely eliminated (Table 6). There are stereochemical grounds for rejecting the use of double-strand DNA templates, proposed by Stent (cf. ref. 55). Thus, single-strand coding between DNA and RNA is more likely to be associated with unicellular organisms and short generation times, whereas, in metazoan cells, double-strand coding by a Zubay cipher is associated with diploid and polyploid cells and longer generation times.

(5) It may be possible, through single-strand coding, to reconcile the co-existence of wide differences in DNA composition in certain micro-organisms (cf. ref. 79) with the known similarity of their protein, as both the DNA strands are theoretically able to act as the genetic templates. There is the interesting contrast that the DNA base ratios of animals and plants are remarkably uniform[11,80].

(6) The presence in ribosomes of histone-like proteins which are latent ribonucleases (and possibly deoxyribonucleases[48]) may provide a mechanism for protecting template RNA while suppressing 'free' RNA of different base composition. In particular, this selective action could prevent complementary polynucleotide from achieving any permanence.

(7) Three distinct histone fractions with ribonuclease activity are found in the nuclei of human and guinea pig cells, while there are only two in the cytoplasmic ribosomes. It seems possible that certain nuclear histones suppress RNA template function and thereby control the differentiation of cells in higher organisms, as originally proposed by Stedman and Stedman[31].

(8) Virus nucleic acids in their duplex forms are usually found to have base compositions which are the 'antithesis' of the corresponding nucleic acids of the host cells. This property may give them immunity to most of the depolymerases of the host cells, while, at the same time, ensuring their replication by association with a particular histone in the host cell. It may be significant that the RNA viruses so far detected only infect animal and plant cells, in which the DNA is always linked to histone, in contrast to the reported absence of the histone moiety in bacterial cells[81,82].

(9) Recently, the discovery that animal viral RNA (Sanders, F. K., personal communication) is first synthesized within the nuclei of the host cells suggests that it may find there 'activating' histones and possibly replicate by single-strand coding through a DNA template as suggested by Rich[54]. Conversely, it is significant that, when DNA bacteriophages infect *Escherichia coli*, an RNA distinct from host cell RNA and similar to phage DNA[83,84] is formed before any DNA of the progeny can be detected.

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Note added in proof. Yčas and Vincent[85] have compared the specific activities of the 2′,3′ and 5′ forms of nucleotides obtained from RNA extracted from yeast cells with boiling 2 *M* sodium chloride. The pattern of asymmetry of labelling with phosphorus-32 in both forms of nucleotides is in general similar to the pattern observed in the 2′,3′-nucleotides from nuclear and ribosomal RNA of *HLM* cells at short labelling times. Analysis of their results leads the authors to conclude that there is, in their material, a small fraction of RNA with a high rate of turnover which is closely similar in base composition to that of yeast DNA. Their suggestion that this RNA might be a primary gene product is compatible with its formation by the Rich[55] or Stent[52] coding systems for RNA template formation.

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A TRANSATLANTIC COMMUNICATION EXPERIMENT VIA *ECHO* I SATELLITE

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THE *Echo* I satellite, launched on August 12, 1960, by the National Aeronautics and Space Administration of the United States, was originally a sphere 100 ft. in diameter, coated with vapour-deposited aluminium to provide radio wave reflectivity of 98 per cent up to frequencies of 4,000 Mc./s. The purposes of the experiment were, first, to check various systems of modulation for 'passive' communications, and, second, to investigate scintillations (which arise in the actual process of reflexion from the satellite), Faraday rotation, fading and polarization

The experiment that we describe here involved communication using continuous wave transmissions only; it was performed on August 18 between Holmdel (New Jersey) and Issy-les-Moulineaux (France). A few days later a modulated signal was received at Jodrell Bank (Great Britain) with the big antenna.

A description of the equipment used by us is given elsewhere[1,2]. We shall give only the numerical characteristics of each component. The parabolic antenna (3·3 m. diameter) is not steerable automatic-

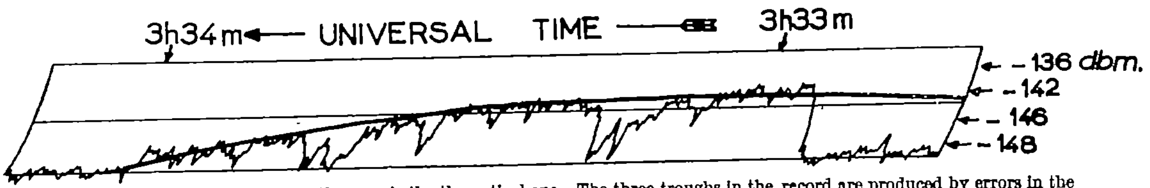

Fig. 1. Amplitude. The smooth curve is the theoretical one. The three troughs in the record are produced by errors in the frequency tracking

ally, so it was pointed to some definite direction before each pass. A flexible air-dielectric cable (100 ft. in length, 1·6 db. loss) was used to join the antenna to the parametric amplifier. The latter was operating in the degenerate mode, and gave an over-all receiver noise figure (measured with respect to 290° K.) less than 5 db. After mixing, the signal is fed to a phase-lock receiver[2,3] which reduces the effective band-width to about 20 c./s., but nevertheless is able to track variations of frequency produced by the Doppler effect. The threshold of the lock-in system is 7 db. above noise-level.

It is now easy to compute the power at amplifier input corresponding to the weakest signal which can be recorded. We have:

kTB =	– 161 dbm.
Noise figure	5 db.
Threshold	7 db.
Total $P_{\text{min.}}$ =	– 149 dbm.

This was in fact the figure measured with a calibrated signal generator (see Fig. 1).

Now we must compute the expected power P available at the focus of the antenna. This power is given by the following equation:

$$P = \frac{P_E G_E D_S^2}{16 l_1^2} \cdot \frac{\eta A}{4\pi l_2^2} \qquad (1)$$

in which P_E, transmitter power, is 9×10^3 W.; G_E, gain of the transmitting antenna (estimated), is 44 db.; l_1, distance between satellite and transmitter, is 4,000 km.; l_2, distance between satellite and receiver, is 3,300 km.; D_S, satellite diameter, is 30·5 m.; A, area of receiving antenna, is 8·55 m.^{2}; η, receiving antenna efficiency, is 0·47. With these data, equation (1) gives:

$$P = -136 \text{ dbm.}$$

However, three additional facts must be taken into account. First, the transmitted polarization was circular, whereas our primary feed was linearly polarized, thus losing 3 db. Secondly, the transmitter – satellite – receiver angle was 81°, which is very near the value 90° for which the scattering coefficient is reduced by 1·3 db. compared with the isotropic case[4] (for which we have assumed a cross-section of $\pi D_S^2/4$ rather than πD_S^2 as is assumed in ref. 4). Finally, we have to subtract the 1·6 db. loss of the cable.

Thus the maximum available power at the amplifier input is:

$$P_{\text{max.}} = -142 \text{ dbm.}$$

The difference between $P_{\text{max.}}$ and $P_{\text{min.}}$ is only 7 db., and, due to the great number of variables on which it depends, this very narrow margin can explain why, among the twelve trials that we made, only one was completely successful.

Results

In this particular trial, the antenna axis had an elevation of 15° ± 1° and an azimuth of 277° ± 1° (north to east). The signal was received for 1 min., from 3h. 32m. 56s. to 3h. 33m. 56s. U.T. This duration was about half what was hoped for, the discrepancy being due to instrumental difficulties. Because of the difference between the actual frequency of the transmitter (≃ 960·045 Mc./s.) and the value originally announced (960·050 Mc./s.), we wasted time looking for the signal around a false frequency.

The amplitude recording is given in Fig. 1. Noise is superposed above the monotonically decreasing signal. The lock-in system fell out of lock three times, and this fact explains the three troughs in the record.

Simultaneously, the frequency was printed every 2 sec.; Fig. 2 gives the plotted results. The stability of our frequency standard was better than 1 part in 10^8 a day.

Comparisons

(a) *Orbital parameters.* In order to make some comparison with the theoretical prediction, we have computed the position, distance, and Doppler effect from 3h. 30m. to 3h. 35m. We assumed a Keplerian orbit, with both the right ascension of the ascending node and the argument of the perigee varying linearly with time. Orbital parameters were taken from the *Space Track Bulletins*, the accuracy of which was very poor in the early part of the life of *Echo* I. (For example, the time of nodal crossing for revolution No. 70, given in *Bulletin* 3, was 3h. 00m. 55s.;

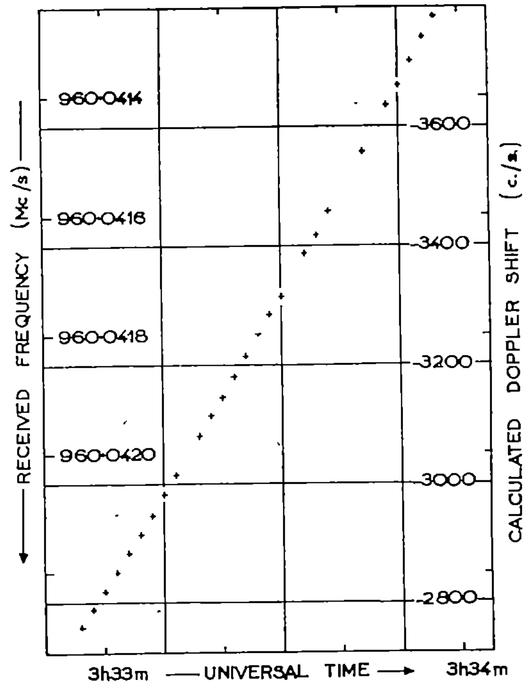

Fig. 2. Frequency. Only the measured points are shown: for comparison with the theoretical curve, see Fig. 3

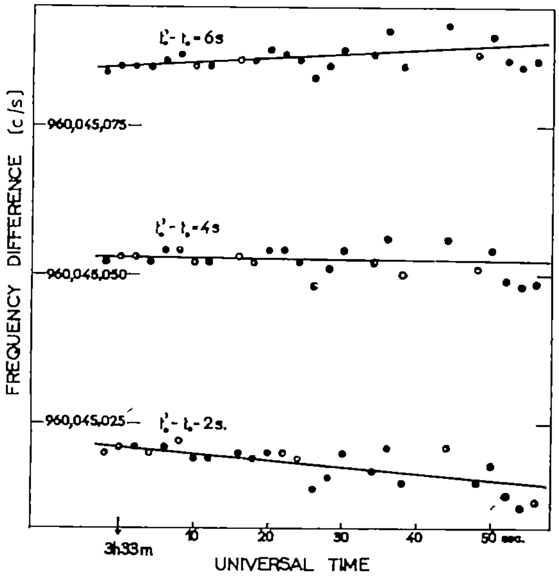

Fig. 3. Difference between the received frequency and the calculated Doppler shift, for three possible values of the time of nodal crossing. This difference is equal to the frequency of the transmitter. If this one had good stability, we must choose $t_0 = t'_0 - 4$s., and therefore $f_0 = 960\ 045053$ Mc./s.

the same time, computed by extrapolation from *Bulletin* 4, was $t'_0 = 3$h. 00m. 31s., and the actual time was $t_0 = 3$h. 00m. 27s. ± 2s., as will be seen below.) We used for our computations the value t'_0, and corrected it, after the event, so as to obtain the best fit between the experimental and theoretical curves; this parameter is the most important one, since, for a duration of 5 min., the others give only a correction of a higher order.

The correction was made by two independent methods. The first method consists in comparing the computed Doppler curve with the observed one, and in seeking the time-shift that gives the best fit between them. Fig. 3 shows the difference between observed frequency and calculated Doppler shift for three possible values of $t'_0 - t_0$, namely, 2s., 4s. and 6s. We see that the values of 2s. and 6s. both imply a considerable drift of the transmitter frequency, of the order of 4 c./s./min. If, as we suppose, the transmitter frequency had in fact good stability (better than 10^{-8} a day), we must choose $t'_0 - t_0 = 4$s.

The second method consists in computing azimuth and elevation for the revolutions $n = 55, 56, 57, 80, 81, 82$, and in comparing them with the optical data (kindly communicated by Prof. P. Muller) for these revolutions; cloudy weather prevented Prof. Muller from observing on the particular night of our experiment.

For each revolution the curves fit well (within ± ½°) provided we suppose that there is a difference between the chosen time t'_n and the actual one (t_n). The differences $t'_n - t_n$ are plotted with respect to n in Fig. 4. A parabola drawn through the six points on this plot gives also $t'_0 - t_0 = 4$s. A parabola is involved because not only was an erroneous value chosen for the nodal period, but also this period was assumed to remain constant, whereas in fact it decreased with increasing time.

(*b*) ***Frequency curve.*** From the best fit of the two Doppler curves, we are able to compute the transmitted frequency:

$$f_0 = 960\ 045\ 053 \text{ c./s.}$$

The error Δf_0 in our measurement of f_0 can be calculated by assuming a maximum error $\Delta t_0 = \pm 2$ sec. in the time of nodal crossing. The slope of the frequency curve being about 1,050 c./s./min., the resulting Δf_0 is ± 35 c./s. as we can also see on Fig. 3, to which we must add the error of our frequency standard, so that:

$$|\Delta f_0| \leqslant 45 \text{ c./s.}$$

Examination of the points on Fig. 3 shows that their scatter is less than ± 4 c./s., and increases when the signal strength decreases (see also Fig. 1); this is a property of the phase-lock system. When compared with the scatter generally obtained in measurements of the frequency of satellite-borne oscillators[1], the present superiority of ground-located transmitters is obvious.

(*c*) *Amplitude recording.* Now, we will turn our attention to the amplitude recording. First the maximum strength of the received signals is − 142 dbm., that is, it is very close to the predicted strength (within ± 1 db., which was the limit of accuracy of the calibration). Thus the reflexion seems to occur as in the idealized case; this implies a good sphericity of the balloon and no significant deterioration of the coating.

Secondly, the decreasing strength of the signal is fully explained by the passage of *Echo* I through the reception pattern of the antenna, thus excluding large fading of period less than ≃ 1 min. This can be shown by computing azimuth and elevation and drawing the path as the satellite crosses the antenna pattern (Fig. 5). From this curve, we can deduce, with respect to time, the theoretical strength of the signal, and compare it with the experimental one; we do not make any allowance for the variation of l_1 and l_2, since their squared product has a negligible variation during 1 min. (< 0·2 db.). The best fit (smooth curve of Fig. 1) is obtained when we suppose not only that $t_0 = t'_0 - 4$s., but also that our antenna beam was pointed towards *B* (Fig. 5) rather than towards *A*. This remains well within the accuracy of ± 1°. (A change from *A* to *C* would not affect very much the shape of the curve.)

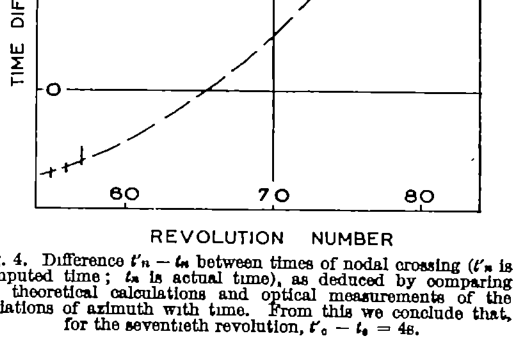

Fig. 4. Difference $t'_n - t_n$ between times of nodal crossing (t'_n is computed time; t_n is actual time), as deduced by comparing the theoretical calculations and optical measurements of the variations of azimuth with time. From this we conclude that, for the seventieth revolution, $t'_0 - t_0 = 4$s.

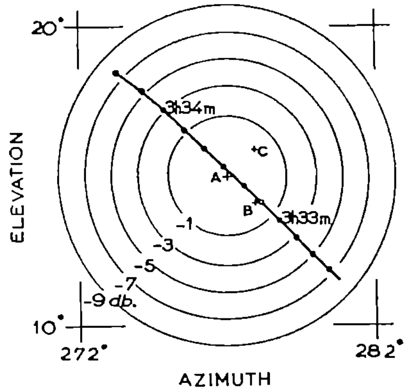

Fig. 5. Crossing of the antenna beam by *Echo* I (points every 10 sec.). *A* is the theoretical beam axis, *B* is the beam axis giving the best agreement with the experimental amplitude record. (The great circles are to be shifted in the same manner)

Fig. 5 also shows that, if we had had a receiving antenna with a beam width of 1° (as for the transmitting antenna at Holmdel), an error of tracking of ½° in angle, or of 5s. in time, would have given a loss of 3 db. (Losses of 5 db., because of lack of tracking accuracy, were found by another observer[5].)

Conclusion

This experiment, among many others, has proved the success of the *Echo* I project; in particular, it showed that the reflectivity of the coating is good, and that the received signals are free from rapid fading.

We have also seen the necessity of a big steerable antenna, in order not only to have better gain (say, 10 db. more), but also to avail oneself of the low temperature of the sky. But the beam-width of the antenna must not be too small, unless very good means of tracking are available.

From the point of view of communications, we must pay attention to the very unfavourable power balance in the use of a passive satellite; even with elaborate equipments, the band-width capabilities are limited to a few voice channels. But the simplicity of the balloon, together with the value of the many scientific results obtained with *Echo* I, argue for more elaborate tests of this kind.

Thanks are expressed to the National Aeronautics and Space Administration, which gave us much information, and to the Bell Telephone Laboratories, which supplied the transmissions to the balloon at the time when it was visible from both Europe and America.

We are indebted to Prof. P. Muller of Meudon Observatory for his optical observations, to Mrs. Epfelbaum for her constant help in the computations, and to the Direction des Etudes et Fabrications d'Armements for the loan of an electronic computer.

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[3] Jaffe, R., and Rechtin, E, *Inst. Rad. Eng. Trans. on Information Theory*, 1, 66 (1955).

[4] Vea, T. H., Day, J. B., and Smith, R. T., *Proc. Inst. Rad. Eng.*, 48, 620 (1960). See also comments about this paper in *Proc. Inst. Rad. Eng*, 48 (September 1960) by Kennaugh, E. M., Morgan, S. P., Weil, H. (p. 1781), and Laugan, N. A. (p. 1782).

[5] *Aviation Week and Space Technology*, 73, No. 8, 30 (August 22, 1960).

OBITUARIES

Prof. J. S. Rankin

Dr. James S. Rankin, Freeland professor of natural philosophy in the Royal College of Science and Technology, Glasgow, died on November 16 in his sixty-seventh year.

Dr. Rankin joined the staff of the Natural Philosophy Department of the College in 1919, after a few years of postgraduate experience in industry, and except for the period 1943–45, when he was seconded to the University of Istanbul to occupy the chair of theoretical mechanics, his service to the College remained unbroken. He was appointed to the Freeland chair in 1942, and under his leadership the Department was a most stimulating place in which to work. His research interests extended over a wide field, his most notable work being concerned with the phenomenon of magnetostriction and, in particular, with attempting to explain the changes in the magnetostriction of steel resulting from various degrees of tensile over-strain.

One of his greatest achievements lay in the development of his course leading to the associateship of the College in applied physics. None of his predecessors had thought of the Department as other than an ancillary one for the engineering and other applied science courses in the College. In 1947 he set about his task of building up a school of applied physics which would have a recognized place in the higher aspects of technology. It was characteristic of his vision and determination that he succeeded, and young graduates from his school are now helping to bridge the gap that exists in industry between the 'pure' physicist and the engineer.

Among his other activities he was a governor of the West of Scotland Agricultural College, a member of the Vice-Chancellors' Committee on Hungarian Students and an ex-chairman of the Scottish Branch of the Institute of Physics and the Physical Society.

He had great gifts which he put to fine use in the service of the College. A devoted teacher, he will long be remembered by generations of students who came to know the warmth of his friendship.

J. W. Sharpe

Mr. E. C. Fieller

Edgar Charles Fieller, officer-in-charge of the Statistical Advisory Unit of the War Office, died suddenly on December 1. He was fifty-three years of age.

A prominent member of the Royal Statistical Society, he had served as a vice-president, several terms as a member of Council, as secretary, then chairman of the Research Section, and as a member of several committees. In 1960 the Society honoured him by the award of the Guy Medal in silver. He was for four years secretary of the British Region of the Biometric Society.

As a young man, after taking the Mathematical Tripos as a scholar of King's College, Cambridge, he was for a time a member of Prof. Karl Pearson's staff. This began an association with the School of Statistics at University College, London, which was renewed in later years through his membership of the University board of studies and by his appointment as an examiner.

His subsequent experience with Messrs. Boots led him into the fields of market research and bioassay, in which he made several original contributions; one of these, a method for obtaining confidence limits for a ratio, is now well known as Fieller's Theorem.

Fieller served during the Second World War in the Operational Research Group of Fighter Command, R.A.F., part of the time as deputy to the officer-in-charge. After the War he joined the National Physical Laboratory in charge of the Statistics Group of the newly formed Mathematics Division. This Group was eventually transferred to the Ministry of Supply and, on the dissolution of that Ministry, to the War Office.

While on leave of absence from the Ministry of Supply, he fulfilled an assignment in Turkey for the Food and Agriculture Organization of the United Nations. There he conducted a training course in sampling methods which enabled a national survey to be made. He made many friends in Turkey, and his work there will have lasting effect.

In later years, when Fieller became a member of the International Statistical Institute, he made a particular point of seeking the acquaintance of the mathematical statisticians of other countries. These personal friendships must have made a considerable contribution to international understanding.

Fieller's published work of earlier years on probability problems and on bioassay was supplemented later by a paper on fiducial limits; in this, with his usual caution, he pointed out the consequences and paradoxes of a theory while reserving judgment on controversial points.

The pleasure he took in social contacts, his understanding of others and his fund of anecdotes, made him a good companion who will be missed in many places.

E. D. VAN REST

NEWS and VIEWS

Entomology at the British Museum (Natural History): Dr. W. E. China, C.B.E.

DR. W. E. CHINA, who retires from his post as keeper of the Department of Entomology in the British Museum (Natural History) at the end of January, is a world authority on the insect order Hemiptera. During his thirty-nine years of service on the staff of the Department he has published more than two hundred papers on the structure, taxonomy, evolution and zoo-geographical distribution of this group of insects. After his retirement he intends to return to this work, which his administrative duties as keeper have interrupted. He became deputy keeper in 1945 and was largely responsible, under the keeper, Mr. N. D. Riley, for the rehabilitation of the Department of Entomology after the war-time bombing and the return of the National collection of insects, much of which had been evacuated. On the completion of the new building in 1951 he was concerned with the keeper in organizing the re-allocation of the entire entomological collection. He was appointed keeper in 1955. Dr. China is a foreign member of the Societas pro Fauna et Flora Fennica and corresponding member of the Société Entomologique d'Egypte. In 1959, he was appointed assistant secretary of the International Commission on Zoological Nomenclature.

Engine Research, Ministry of Aviation: Mr. Peter Lloyd, C.B.E.

MR. PETER LLOYD has been appointed director general engine research and development, Ministry of Aviation, in succession to Mr. R. H. Weir, who is now director of the National Gas Turbine Establishment, Pyestock. Born in 1907, Mr. Lloyd went from Greshams School, Holt, to Trinity College, Cambridge, in 1925, where he took the Natural Science Tripos, Parts 1 and 2 (chemistry). After a year's research under Dr. (now Sir) Eric Rideal and a year in the German chemical industry, he was with the Gas Light and Coke Co. from 1931 until 1940 in charge of research on the industrial utilization of gas. In 1940 he joined the Royal Aircraft Establishment working first for Prof. G. T. R. Hill in the Air Defence Department at Exeter, and then in 1941 under Hayne Constant and W. R. Hawthorne in the turbine division of the Engine Department. With that team he transferred to Power Jets, Research and Development, Ltd., at Pyestock, in 1944, and soon after that body became the National Gas Turbine Establishment he became a deputy director. During the first part of this period his work was mainly concerned with combustion researches including studies of spontaneous ignition characteristics at high temperatures and the evaporation and burning characteristics of liquid fuel drops in high-speed air streams. Latterly he assumed a more general responsibility for Pyestock's research on aerodynamic, mechanical and metallurgical problems. Following two visits to Australia in connexion with the work of the Commonwealth Advisory Aeronautical Research Council, he was posted to Australia in 1960 as temporary head of the Ministry of Aviation Staff, during the period of recent negotiation with the Australian Government on space research. Peter Lloyd is a mountaineer who has taken part in three Himalayan expeditions, including the 1938 attempt on Everest, in the course of which he made the first comparative trial between open- and closed-circuit equipments. As a member of the Himalayan Joint Committee of the Alpine Club and the Royal Geographical Society he was closely concerned with the assaults on Everest, being responsible for the provision of the oxygen equipment used in the successful assault.

Monsanto Chemicals, Ltd.: Senior Scientist

DR. I. S. WILSON has been appointed senior scientist to Monsanto Chemicals, Ltd. This appointment is part of a Monsanto scheme under which outstanding scientists on the company's staff may receive proper recognition of their status while continuing to specialize in scientific or technical work rather than accepting increased administrative responsibility. In his new capacity Dr. Wilson will be free to devote his whole time to research on effluent treatment and the microbiological breakdown of organic compounds.

The Leopoldina German Academy of Sciences : Elections

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members, in the sections indicated : *Mathematics*, Prof. Emil Artin (Hamburg) ; Prof. I. R. Safarevic (Moscow) ; Prof. B. L. van der Waerden (Zurich). *Pathology and Anatomy*, Prof. Gunter Bruns (Halle) ; Prof. Hermann Chiari (Vienna) ; Prof. Wilhelm E. Ehrich (Philadelphia) ; Prof. Andreas Werthemann (Basle). *General Biology*, Prof. Th. Dobzhansky (New York) ; Prof. Ake Gustafsson (Stockholm) ; Prof. Herman J. Muller (Bloomington) ; Prof. I. I. Schmalhausen (Moscow) ; Prof. Josef Straub (Cologne). *Zoology*, Prof. Robert Mertens (Frankfurt on Main).

Indian Institute of Science : Golden Jubilee

ON glancing through the Golden Jubilee Research Volume of the Indian Institute of Science (Bangalore, India, 1959), one is agreeably impressed by the diversity and range of scientific endeavour in India. As one would expect in a country developing its technology, applied science is given certain emphasis. There are articles on mineral flotation and extraction, turbomachines, silkworm nutrition, alloys, electronics research, soil fungi and concrete curing. But in more fundamental areas, too, research has been contributed —organic chemical syntheses, chemical kinetic investigations, derivation of expressions for compressible fluid-flow in the presence of magnetic fields, Hermite orthogonal functions and so forth. Science in India appears to have a character of its own—somewhat refreshing in this world of fading individuality. One article, for example, describes research on an Ayurvedic medicinal preparation called 'drakshasava'. This fermentation product has been of wide popularity in the country for a long time. It is claimed to cure a range of maladies like indigestion, cholic, insomnia, loss of appetite, phthisis and 'excessive debility'. An elegant microbiological evaluation of the drug was carried out. It was found only to support the growth of the micro-organism, *Saccharomyces cerevisiae*. Also, addition of vitamins and amino-acids did not stimulate growth. Except for sugars and trace elements, the drug did not contain essential growth factors—its only tonic effect appeared to be due to its alcoholic content. Pharmacological examination would be required to reveal the presence of very small quantities of ingredients which do cause the healing properties claimed. Nevertheless, establishing the use of microbiological technique in this way does provide scope for probing into these age-old medicines and disclosing their mysteries.

Mathematics in South Asia

THE report of the Second Conference on Mathematical Education in South Asia, held at Bombay in January 1960, has now been issued (Pp. xxiii+205 ; Tata Institute of Fundamental Research, Bombay ; September 1960). Prof. Chandrasekharan, of the Tata Institute, presided over the Conference, and in his presidential address he urged the Conference not to think entirely of technical problems but to remember also that the great need in South Asia is the construction of a social and educational environment conducive to the growth of creative scholarship. In the report, the invited addresses are published in full, and the proceedings of the working parties are summarized. The accounts of the progress of mathematical education in Malaya, Indonesia, Thailand, Pakistan and Ceylon show what efforts have been made to implement the suggestions put forward by the first Conference (February 1959).

While emphasizing the vastness and complexity of the task, the addresses are optimistic, confident that in a short time significant progress towards national mathematical education culminating in vigorous schools of research will have been made. The other invited addresses, by mathematicians from Europe, North America and Asia, deal mainly with algebra and geometry, in the upper school, at the undergraduate-level, and in the first postgraduate year. Generally, stress is laid on the axiomatic approach and the basic importance of modern linear algebra, founded on a school knowledge of the elements of vector algebra and of sets of linear equations. Several of the addresses advocate an earlier introduction, in the universities, of modern abstract and general methods, on the grounds that students of to-day are not afraid of abstractions and can thus derive full advantage from an early acquaintance with general methods. The reports of the working groups preserve the flavour of lively and provocative discussions.

The Manchester Literary and Philosophical Society

VOLUME 102, 1959–60, of the Memoirs and Proceedings of the Manchester Literary and Philosophical Society well justifies Sir Cyril Hinshelwood's commendation of the Society in opening its new buildings in September (Pp. 81 + xliii. (Manchester : Manchester Literary and Philosophical Society, 1960.) 31*s*. 6*d*.). It includes the presidential address of Mr. C. E. Young on the growing importance of organo-metallic chemistry ; Dr. G. E. R. Deacon's Clayton Memorial Lecture on the scientific exploration of the oceans ; Prof. R. D. Waller's lecture on Danilo Dolci and his sociological work in Sicily and southern Italy ; Sir Gordon Sutherland's lecture giving some comparisons of British and American Universities ; and Prof. C. F. Carter's assessment of the practical value of the economist in government and in business in which he suggests that the Minister for Science should encourage a joint technical and economic appraisal of the opportunities for State-encouraged scientific development.

Irregularities in Spelling

BACKWARDNESS in the basic subjects has been the subject of considerable unfavourable comment by industrialists and others since the War. Among the causes of backwardness in reading, English spelling has long figured as an important factor. Spelling reform has been increasingly mooted since George Bernard Shaw's bequest for this purpose, and recently, the National Foundation for Educational Research in England and Wales in conjunction with the University of London Institute of Education agreed to undertake research into the alphabet form. An important preparatory step has been Dr. W. R. Lee's scientific study of the part played by spelling irregularity as a contributory cause of backwardness in reading. The results of his investigations have now been published under the title "Spelling Irregularity and Reading Difficulty in English" (Occasional Publication, No. 2 : Pp. i+74. London : National Foundation for Educational Research in England and Wales, 1960. 10*s*.). It has been maintained that, if each sound were always represented by the same letter, reading proficiency and reading habits would be achieved more easily. This study examines this hypothesis against experience at home and abroad, and follows this up with objective testing in experimental situations. The decisive conclusions should form an invaluable aid to teachers, administrators and all concerned with progress in education.

Foot-and-Mouth Disease in Madras

IN the combined report of the King Institute of Preventive Medicine, Guindy, and the Government Analyst, Madras, for the year 1957–58, information is provided about the problem of foot-and-mouth disease in Madras State (Report of the King Institute of Preventive Medicine, Guindy, and Report of the Government Analyst, Madras, for the period from 1st April, 1957, to 31st March, 1958. Pp. i+121. Guindy, Madras: King Institute of Preventive Medicine, 1960). The conclusions of a committee set up to examine the position were that: foot-and-mouth disease is endemic in the whole Madras State and occurs year after year; the carrier state of the infected animals is quite long and lasts a year; it is not practicable to select virus-free animals for vaccination; and that with the facilities available at the Institute, every possible precaution has been taken to minimize the infection and its spread. The committee was of the opinion that controlled measures have become all the more important, as prophylactic vaccines against foot-and-mouth disease are not available in India. It is also not practicable to import vaccines from abroad as they are very costly and protection against local strains cannot be assured. The report contains detailed information about all measures taken to safeguard public health in the State.

A New Frog from Brazil

AMONG the specimens in a small collection of frogs sent to Coleman J. Goin of the University of Florida was a single individual of a striking little *Hyla*. It has been named *Hyla bokermanni*, n. sp. The specimen was an adult female and has small rounded vomerine tooth patches and small rounded dorsolateral stripes on a chocolate-brown background. It is perhaps most closely allied to *H. parviceps*, from which it differs in its smaller size, shorter legs, smoother skin, and in having a pattern of dorsolateral stripes as well as differences in detail of pattern of the thighs. From *H. microps*, which may also be a near relative, it likewise differs in its small size, shorter legs and in the pattern of dorsolateral stripes (*Ann. Mag. Nat. Hist.*, 2, No. 24; December 1959).

Fecundity of the Nile Mormyrid, *Hyperopisus bebe* (Lacepede)

ATTENTION has recently been directed to the importance of the fish fauna of the Nile as a valuable source of protein food. Experimental stations have been constructed for the breeding of some of the economically important species of the Nile fishes. Work on the fecundity of the Nile fishes would, therefore, be of importance for such projects and for a general control scheme for the maintenance and increase of the productivity of the rivers, but except for the observations made by Kenchington in 1933 on the fecundity of the Nile perch *Lates niloticus* (Linnæus 1762) no one has previously examined this problem. G. Nawar has now investigated the fecundity of *Hyperopisus bebe* (Lacepede) (*Ann. Mag. Nat. Hist.*, 2, No. 22; October 1959). The fecundity, defined as the number of eggs produced by the female during the breeding season, was determined through the study of 62 females. It was found to vary in the range 2,030–33,060, provided that the fecundity is calculated on the basis of counting the whole number of ova per ovary. A correlation between fecundity, length and weight of the specimen was observed.

New Virus Isolated from Culicine Mosquitoes

DURING April 1958, an outbreak of febrile illness of unknown ætiology occurred among the residents of Germiston, a town near Johannesburg. As it was considered that this outbreak might have been due to an arthropod-borne virus, mosquitoes were collected with the intention of isolating the causal agent. In the centre of Germiston, which is a highly industrialized town, is a small lake with an adjoining golf course and park. On the edge of the lake is a dense reed-bed with a heronry in which large numbers of cattle egrets (*Bubulcus ibis*) roost and breed. At the time the mosquito collections were made, the heronry contained hundreds of fledgling cattle egrets. Associated with the reeds and surrounding trees were lesser numbers of black-headed herons, sacred ibises, coots, moorhens, reed warblers, weavers and doves. Mosquitoes were collected on the edge of the lake in proximity to the reeds. From *Culex* (*Neoculex*) *rubinotus* Theobald mosquitoes collected at Germiston, three virus strains were isolated. Two of these strains were identical and have been shown to belong to the Bunyamwera group of arthropod-borne viruses. B. M. McIntosh, R. H. Kokernot and H. E. Paterson report in the April issue of the *South African Journal of Medical Science* that they have now isolated a third virus strain which has been shown to be unrelated immunologically to many arthropod-borne viruses. Accordingly, the name Witwatersrand virus has been proposed, which has reference to the geographical area where the mosquitoes were collected and from which the agent was isolated.

Essay Competition on Corrosion Science

THE Corrosion Group of the Society of Chemical Industry is organizing a competition designed to encourage persons who are still in the early stages of their career to take an interest in corrosion science. The competition will take the form of an essay, and persons aged not more than 30 years are invited to present an essay or paper on any aspect of corrosion of metals and its prevention. A prize of 25 guineas will be awarded. The closing date for receipt of entries is March 31, which should be addressed to Corrosion Group Essay Competition, c/o Society of Chemical Industry, 14 Belgrave Square, London, S.W.1, whence further information can be obtained.

"Effect of Corona Discharges on Polyethylene"

REFERRING to the communication under this title published in *Nature* of November 12, 1960, p. 577, Mr. L. R. Hougen states: "Further work has revealed that the identification of the alcohols in the gas chromatogram (Fig. 1) is incorrect, due to false labelling of the marker compounds, which was beyond my control. A correct interpretation of the gas chromatogram indicates that the distribution of breakdown products between the different carbon numbers is statistical, and that the largest peak represents C_{20} secondary alcohols, which suggest that a free-radical mechanism may play an active part in the breakdown reactions".

The Night Sky in February

New moon occurs on Feb. 15d. 08h. 11m. u.t.; full moon does not occur this month. The following conjunctions with the Moon take place: Feb. 2d. 07h., Regulus 1° N.; Feb. 13d. 06h., Jupiter 4° S.; Feb. 13d. 07h., Saturn 3° S.; Feb. 18d. 11h., Venus 7° N.; Feb. 22d. 20h., Aldebaran 0·2° S.; Feb. 24d. 17h., Mars 8° N. Jupiter is in conjunction with Saturn on Feb. 18d. 15h., Jupiter 0·2° S.; this phenomenon occurs once every twenty years, and on this occasion the approach is unusually close, but conditions for observation are unfortunately not favourable. There is a total eclipse of the Sun on February 15, visible as a partial eclipse at Greenwich. The eclipse will be total along a track passing through southern France, northern Italy, the Balkans and parts of the U.S.S.R., where there will be a maximum duration of 2m. 45s. Seen from Greenwich, the partial eclipse begins before sunrise, attains its maximum phase of 0·93 at 7h. 42m., and ends at 8h. 47m. This is the largest eclipse to be seen from Greenwich since 1927 and until 1999. Mercury will be visible as an evening star during the first half of the month. Venus is an evening star, favourably placed for observation. Venus sets at 21h. 20m., 21h. 45m. and 21h. 50m. on February 1, 15 and 28, respectively, more than 4 hr. after the Sun. During the month its distance from the Earth decreases from 63 to 44 million miles, the visible portion of the apparent disk from 0·499 to 0·315, and its stellar magnitude from −4·1 to −4·3. Mars sets at 6h. 00m., 5h. 10m. and 4h. 25m. at the beginning, middle and end of the month, respectively. Mars is very conveniently situated for observation. It is at a distance of 80 million miles from the Earth on February 15, and its stellar magnitude is −0·1. Jupiter and Saturn are too close to the Sun for easy observation; they are only observable in the twilight before sunrise. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Feb. 10d. 5h. 19·7m., 24 Sco. (*R*); Feb. 22d. 21h. 18·8m., 89 Tau. (*D*); Feb. 23d. 18h. 01·6m., 111 Tau. (*D*); Feb. 26d. 22h. 19·1m., 5 Cnc. (*D*). *D* and *R* refer to disappearance and reappearance, respectively.

Announcements

Dr. Rudolf L. Mössbauer has been awarded the 1960 Research Corporation Award by the California Institute of Technology and Research Corporation for his work on the production and detection of gamma rays. Dr. Mössbauer is at present on leave of absence at the California Institute of Technology from the Institute of Technical Physics, Munich.

Among the recent changes in administration and organization of the Akers Research Laboratory, Imperial Chemical Industries, the following appointments are announced: Dr. P. W. Brian, to become associate research manager of the Pharmaceutical Division and to be in general charge of the Laboratory; Dr. J. Chatt, to become group manager of the Heavy Organic Chemicals Division. Dr. Chatt will continue in charge of the Organic Chemistry Department.

The Automatic Programming Information Centre, Brighton Technical College, is organizing a course in Algol 60 during April 5–6. Further information can be obtained from Richard Goodman, Automatic Programming Information Centre, Brighton Technical College.

The annual general meeting of the Scottish Branch of Aslib is to be held at the National Engineering Laboratory, East Kilbride, on February 3. Further information can be obtained from the honorary treasurer, J. R. K. Pirie, M.E.R.L. (Aslib), East Kilbride, Glasgow.

The Institute of Physics and the Physical Society is organizing a conference on "The Crystal Structures of Organic Compounds" in the Chemistry Department of the University of Glasgow during April 6–7. Further information can be obtained from the Secretary of the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

The Faraday Society is organizing an informal discussion on "Mechanisms of the Gas-Phase Reactions of the Halogens and their Compounds" at the Chemistry Department, University College, Cardiff, on March 28. The programme will be divided into two sections: (*a*) Oxidation processes; (*b*) reactions of elementary halogens. Further information can be obtained from Dr. J. H. Thomas, Chemistry Department, University College, Cathays Park, Cardiff.

A conference is being organized by Research and Control Instruments, Ltd., on "X-ray Analytical Methods" at the Department of Geology, University of Manchester, during March 20–24. Further information can be obtained from the Conference Secretary, Research and Control Instruments, Ltd., Instrument House, 207 King's Cross Road, London, W.C.1.

The Institution of Mechanical Engineers is organizing a symposium on "User Experience of Large-Scale Industrial Vacuum Plant" at the Institution during March 1–2. The symposium is being sponsored by the Joint British Committee for Vacuum Science and Technology. Further information can be obtained from the Secretary of the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

The Department of Scientific and Industrial Research is organizing a two-day national conference on "Science and Industry—the Problem of Communication", which is to be held at the Brangwyn Hall, Swansea, during April 18–19. Further information can be obtained from the Press Officer, Central Office of Information, Park Place, Cardiff, or the Department of Scientific and Industrial Research Press Office, 5–11 Regent Street, London, S.W.1.

An all-day meeting of the Welsh Soils Discussion Group will be held in the Department of Agricultural Chemistry, University College of North Wales, Deiniol Road, Bangor, on February 8, to consider "Glaciation in Wales as related to Soil Profile Development". Further information can be obtained from D. F. Ball, The Nature Conservancy, Penrhos Road, Bangor, Caernarvonshire, or James A. Taylor, Department of Geography, University College of Wales, Alexandra Road, Aberystwyth.

Erratum. We regret that the title of the communication by J. S. Machin and D. L. Deadmore in *Nature* of January 21, p. 223, is incorrect; it should read, "Thermal Stability of $TiOF_2$". Throughout the text the formula '$TiOF_2$' should be substituted for 'titanium dioxide', except where the latter is defined as anatase and in the third paragraph from the end.

THE NUFFIELD UNIT OF TROPICAL ANIMAL ECOLOGY

IN the past few years biologists in general have come to realize the great importance of an intensive study of tropical animals, their ecology, and the conditions of their existence. It is not merely that tropical Nature is a source of invaluable scientific information which is in danger of vanishing through the changing of the face of the Earth by man. It is also that the fauna of tropical nature reserves gives us an opportunity of determining with precision the factors that control both the physiology of individual animals and the size of their populations under conditions as yet relatively unspoilt. We need critical analysis of ecological factors, aided by laboratory investigations, together with the physiological study of what makes tropical species so much more efficient for the production and maintenance of animal substance than species imported from temperate latitudes.

It was with studies of this sort in mind that, on the initiative of Sir Landsborough Thomson and the Zoological Society, in discussions with the Director of the Nuffield Foundation, a proposal to form a Unit of Tropical Ecology was developed and placed before the Foundation. The Foundation has accepted the proposal and given it most generous and substantial support. The University of Cambridge has readily accepted the proposal that the Unit should be controlled from the University.

The immediate objective of the Unit is to maintain one senior and one junior research worker in tropical ecology to work at the Queen Elizabeth National Park, where the Trustees of the Uganda National Parks already have a small field station. The work of the Unit will, however, be controlled from the University of Cambridge, by a Committee under the honorary chairmanship of Prof. C. F. A. Pantin. The Committee will include representatives from the University of Cambridge, the Nuffield Foundation, the Zoological Society of London and the Trustees of the Uganda National Parks. It is an important part of the scheme that, while the field work will be done in Uganda, the research workers will spend substantial periods planning their work and studying their observations in the Zoological Laboratory at Cambridge. It is hoped that as the scheme develops other research workers will be encouraged to attack problems in Uganda, and also that it will lead to young research workers engaged on such problems coming to work at Cambridge. The authorities of Makerere College have already shown great interest in the Unit.

The Committee will be holding its first meeting early this year to consider staff appointments, and sent Dr. Hugh B. Cott to visit the field station in December and to make a first report. The promoters of the scheme have great hopes that the Unit will lead not only to important work on the ecology, physiology and environmental relations of tropical animals, but also to a greater contact between research workers in Britain and Uganda than has hitherto been possible: further, that the attack on animal ecology may later develop into a broader project, including plant ecology and the study of micro-organisms.

BRITISH COMMUNICATIONS SATELLITE PROGRAMME

IN replying for the Government in an adjournment debate in the House of Commons on December 21 on a British communications satellite programme, which had been opened by Mr. A. Neave, Mr. G. Rippon, the Parliamentary Secretary to the Ministry of Aviation, welcomed the practical approach of speeches in the debate. Previously he had pointed out that communications satellites involve the use of a powerful launcher and that the project is thus distinct from the programme based on *Skylark*, the giant programme in the United States, and also the Preparatory Commission set up to study the possibility of establishing a European space research organization. Both British and American experts are confident that the purely technical problems can be solved within the next few years, and Post Office and Ministry of Aviation scientists and engineers are already carrying out joint work on the design of ground stations and satellites as well as of overall system design. This work is concerned with both technical and economic aspects of satellites. While the co-operation of industry will be sought at the appropriate stage, he did not think that there is anything to be gained by setting up a space communications committee at Cabinet level, as suggested by Mr. Neave.

Mr. Rippon pointed out that many different types of system have been proposed, but experts, both in the United States and in Britain, to-day appear to favour a system using active satellites which will carry radio receivers and transmitters, thus receiving, amplifying and re-transmitting signals instead of just reflecting. He promised to bear in mind what Mr. D. Price had said as to the possibilities of such a system.

The study of system design includes that of the type of orbit to be chosen for the satellites, which could be circular or elliptical, close to the Earth or far away. It is possible to choose a particular circular orbit for a satellite at about 22,000 miles from the Earth, and three such satellites could be so placed that one at least is visible and potentially available for communication purposes from every country in the world, except for certain limited areas near the poles. This system has some disadvantages, and it may well be better to have a larger number of satellites in closer orbit, for example, a dozen or two satellites at 5,000 or 6,000 miles, or 50 at 2,500 miles. Whatever orbit is chosen, there are formidable difficulties, and the technical problems to be overcome make estimates of cost necessarily speculative at this stage. Reliable information on the useful life of a satellite is required, and this can only be obtained by experiment, for which the most promising area is the North Atlantic. Mr. Rippon hoped that such tests will take place in the next year or so, and said that Britain envisages putting two or three satellites into polar orbit. He also said that Britain is aiming at about 1,000 channels for an active satellite, but there is really no limit to the number of channels for a passive satellite.

Meanwhile, Britain will continue with construction of the submarine cables to provide for the communication needs of the Commonwealth, for which a programme, costing between £35 and £40 million, has been approved, covering the cable between Canada and the United States in 1961 and Canada and Australia in 1966. The United Kingdom's share will be less, and it may be found that cable and satellite communications are complementary indefinitely. As regards a launcher, the Government has kept in being those aspects of the *Blue Streak* project which can be of use in developing a satellite launcher. The scientific and technological skills have been preserved, and the teams necessary to develop a satellite launcher retained: should such a launcher be developed, what Britain has done and is doing would enable her, or any other participating country, to formulate a programme and put satellites into orbit within the next five or six years. We have already concluded that, if a heavy satellite launcher were to be developed outside the United States or the U.S.S.R., there are substantial advantages for all concerned in doing so in co-operation with the Commonwealth and Europe. Mr. Rippon directed attention to the fact that Britain is keeping in close touch with Australia and Canada and other members of the Commonwealth, and, if the *Blue Streak* proposals are carried to completion, the facilities at Woomera will be used. The Government has also had lengthy discussions with the French Government on the joint development of a satellite launcher based on *Blue Streak*, and these have gone well. It is intended to ask interested countries to join in a conference early in 1961, and in the meantime individual countries are being invited to send their experts here for discussions and to judge for themselves the technical soundness of the British proposals.

FUTURE OF THE COLONIAL AND THE COMMONWEALTH RELATIONS OFFICES

IN answer to questions in the House of Commons on December 13, the Prime Minister said that he is considering, in consultation with the Commonwealth and Colonial Secretaries, the recommendations regarding the future of the Colonial Office and the Commonwealth Relations Office made by the Select Committee on Estimates, and that the subject will be discussed with other Prime Ministers of the Commonwealth at their Conference to be held during March. Meanwhile, the problem of fusion is being studied to see whether some of the advantages of co-ordination can be secured without the disadvantages of combining relations with independent and dependent members in a single Office. In replying for the Government in a subsequent debate in the House of Commons on December 19, on the Select Committee's report, the Colonial Secretary, Mr. Iain Macleod, said that it is clear that the present policy of leading the dependent territories to full independence as early as is compatible with their educational, political and economic development is bound before long to lead to a stage when there is no longer justification for a separate Office, headed by a Secretary of State, to conduct the relations with the few small remaining dependencies. A Commonwealth Service is another matter, and the preference of the independent countries for independent recruitment and for local officers rather than expatriates destroys the basis of a Commonwealth Service. Meanwhile, the burden of work falling on the two Secretaries of State is too great for a merger to be practical for at least a year or two ahead. Moreover, territories which are reaching independence during the next few years will continue to require in their services expatriate officers in professional, technical and administrative posts, and the supply of such officers concerns both the Colonial Office and the Commonwealth Relations Office.

Ultimately, Mr. Macleod thought, the solution might well lie in the bringing together of the two Offices as the Select Committee recommends, but this is not yet practical politics. Meanwhile, the Government has initiated a study of the possibility of creating a joint Department under a Minister responsible to the Colonial Secretary, the Secretary of Commonwealth Relations and the Foreign Secretary to handle under a single direction many of the forms of technical and advisory assistance which Britain provides to overseas countries whatever their status. Mr. Macleod stressed the importance of the contribution which Britain can make in technical assistance through the experience and knowledge of its men and women. It is accordingly proposed to defer consideration of the Select Committee's proposal for a Commonwealth Advisory and Technical Service pending the result of this examination. Mr. Macleod concluded with a warm tribute to the work of the Colonial Service and to the achievements of what is described as British colonialism. Britain can no longer offer many of the members of the Overseas Service the full cover which they have earned, and it is the Government's duty to ensure that everything possible is done to minimize any consequent hardship. He claimed that Britain has the finest organization in the world for providing professional and technical assistance to underdeveloped territories, and the men and women of the Overseas Service and their predecessors have a record of service of which all should be proud.

Mr. Macleod's tribute to the Colonial Service and his concern for the future of the men and women of the Overseas Service echoed what had been freely expressed in the debate, but although there appears to be widespread support for the merger of the two Offices caution was urged from both sides of the House. There is clearly concern that very big changes will be required in the Commonwealth Relations Office itself before it is fit to deal with the new situation and the needs of the newly independent members of the Commonwealth. In fact, there is much greater confidence in the ability of the Colonial Office to provide the advice, the experience, the skill and techniques that will still be required.

HYDROLOGY IN GREAT BRITAIN

FOR several decades a minority of enthusiasts have pressed on the Government the need for an improved hydrological service in Great Britain. Official recognition of this need was recorded recently in the annual report of the Advisory Council on Scientific Policy, where attention was directed to the fact that Great Britain remains weak in research on hydrology. Yet another warning of coming difficulties in meeting demands on water supply in Great Britain was recently given by Sir Herbert Manzoni in his presidential address to the Institution of Civil Engineers. The position shows every sign of becoming serious in a few years time, and in terms of research a great number of problems are involved throughout the whole of the hydrological cycle; ranging more especially from the effects of vegetation on catchment areas to problems of water use, such as waste by consumers and losses from leaks in water mains. Coincident with these warnings and confirming the need for water conservation and a national water policy, the country has had to endure extensive flooding as a result of one of the wettest autumns on record.

Faced with such difficulties it is pleasing to record some recent advances in hydrological research in Great Britain which it is hoped augurs well for future action. The Surface Water Survey of the Ministry of Housing and Local Government now seems well established and is producing an annual report with admirable regularity. The latest edition[1] covers the water year 1958–59, and includes the flow records of 188 gauging stations of which 19 appear for the first time. There is every indication that this number will rise still further in the next issue as many new stations have recently been established and are now in process of calibration. The water-year covered by the 1958–59 volume is of particular interest as it records the effects of the severe drought of the summer of 1959. The rainfall over England and Wales during the period from May to September was only half the long average for 1916–50, and was probably the lowest for more than 230 years. By the end of September many rivers reached new all-time record low levels; indeed, some dried up altogether.

In addition to the responsibility for an annual return the Surface Water Survey was also charged in 1959 with the preparation of hydrological surveys of selected basins. This duty arose from the recommendations of the Sub-Committee on the Growing Demand for Water[2] that hydrological surveys of river basin areas should be carried out, initially in those areas where the expected surplus of water supply over demand is lowest. Two surveys have now appeared: a few months ago the River Great Ouse Basin (Hydrometric Area No. 33)[3] was published, and more recently the River Severn Basin (Hydrometric Area No. 54)[4] has become available. These reports are notable steps forward in the study of Great Britain's water resources, water use and management. There are still, however, another hundred hydrometric areas to be considered if the whole country is to be covered so that a national assessment of the problem now confronting Great Britain may be made.

Both the surveys which have so far appeared attempt a coverage of all the aspects of the hydrological cycle. There is a general study of the area, population, rainfall, geology, principal rivers and hydrology in the first part of each survey. In the second part the hydrometric area is broken down into five geographical sections in each of which the topography, population, hydro-geology, meteorology, gauging stations, water quality, pollution, navigation, flood protection, irrigation, water use and potential water resources are systematically considered. These descriptive paragraphs are followed in each area by details, both in list and map form, of abstraction of water by public undertakings and by industry, discharges of water by public authorities and by industry, and a water balance sheet. These sections are packed with valuable data which will be of immense value to those concerned with the use of the respective water resources. The third part of each report has a summary and forecast of possible future trends.

The River Ouse survey showed that the basin is one of the driest in Great Britain, and with very few natural sites for storage reservoirs available the continued development of surface water is limited. The main problem that gives cause for anxiety is the rising demand for irrigation water. The chief hope for the future lies in a greater exploitation and at the same time proper management of the ground water resources: these appear to be considerable in some parts of the basin.

The River Severn survey, while following the pattern of the River Ouse survey, is more ambitious in that estimates are given ahead to 1980. Covering an area of 4,410 square miles the River Severn Basin reaches from Rugby to Lake Vyrnwy and from Shropshire to the Bristol Channel. It is the largest river basin in Great Britain and has a mean run-off of 2,200 million gallons per day. At present the river basin supports a total water use of 737 million gallons per day for almost 2 million people: of this some 121 million gallons per day are permanently abstracted for use in the area and another 43 million gallons per day are exported without serious consequences as to the remaining quantity or quality of the river water even during prolonged periods of dry weather. The report suggests that maybe another 10 million gallons per day could be taken without noticeable effects during a drought, but that the abstraction of an additional 50 million gallons per day would certainly produce difficulties, while an additional abstraction of 100 million gallons per day might at times dry the river above Bewdley and produce objectionable conditions downstream.

Proposals have already been made by public water undertakings for the construction of works capable of abstracting an additional 100 million gallons per day, so that total abstractions for the mid-1960's could amount to 260 million gallons per day, on top of which has to be added increased direct abstraction by industry and by agricultural interests for irrigation. By 1980 it is anticipated that abstractions could rise to 370 million gallons per day with possibly 64,000 acres under irrigation.

The report makes it clear that these increases can only be achieved by additional storage, controlled use, and a policy of integrated river basin development. The authors reject the extensive use of conventional storage reservoirs and pumped storage schemes and come down in favour of regulating reservoirs, with which, it is maintained, an additional 250 million gallons per day could be guaranteed, an adequate flow in the Severn preserved at all times, much agricultural and urban land protected from flooding, and up to 64,000 acres irrigated in times of drought.

W. G. V. BALCHIN

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[1] Ministry of Housing and Local Government, and Scottish Office. *The Surface Water Year-Book of Great Britain*, 1958–59: *Hydrometric Statistics for British Rivers, together with related Rainfalls, for the year ended 30th September*, 1959. Pp. xiv+92. (London: H.M. Stationery Office, 1960.) 17*s*. 6*d*. net.

[2] Central Advisory Water Committee. Sub-Committee on the Growing Demand for Water. Second Report. Pp. 5. (London: H.M. Stationery Office, 1960.) 6*d*. net.

[3] Ministry of Housing and Local Government. *River Great Ouse Basin: Hydrological Survey* (*Hydrometric Area No.* 33). Pp. 100+9 folding maps. (London: H.M. Stationery Office, 1960.) 12*s*. 6*d*.

[4] Ministry of Housing and Local Government. *River Severn Basin: Hydrological Survey* (*Hydrometric Area No.* 54). Pp. iv+134. (London: H.M. Stationery Office, 1960.) 17*s*. 6*d*. net.

HYGIENE AND PLANT PROTECTION

THE chemical means of plant protection and problems of hygiene have been reviewed by L. I. Medved, director of the Kiev Institute of Labour Hygiene and Occupational Diseases, in a recent issue of *Impact of Science on Society* (10, No. 4, 1960).

The systematic fight against pests and diseases of cultivated plants is not only of great economic importance, but is also socially significant. It has been calculated that the loss of food and technical crops on the farms of the United States due to pests, fungoid diseases and weeds amounts to about 11,000 million dollars a year. Similarly it has been shown that implementation of measures for the protection of plants against pests, diseases and weeds would make an additional quantity of agricultural produce, valued at about 54 billion roubles, available in the U.S.S.R. While the quantity of pesticides produced and employed in agriculture by either of these two countries is considered greater than by any other, and, if they possess enormous reserves for increasing agricultural produce as a result of further use of pesticides, the same opportunities exist in other countries.

The beneficial aspect of the use of poisonous chemicals in agriculture is evident, but the social effects should not be overlooked. All, or almost all, the chemicals employed are biologically active, and in some cases are potentially poisonous for human beings. Investigations by Soviet scientists have shown that the prolonged action on the human organism of various chemical substances, even in insignificant quantities, may lead to a fall in the reactivity of the organism and to a rise in susceptibility to other diseases. In such cases, acute and chronic intoxication does not occur, but a general high level of morbidity is noted.

The problem of the carcinogenic effect of certain chemicals requires special attention. There are indications that carbolineum and its preparations, employed in a number of countries, contain a carcinogenic impurity (benzpyrene). There have been reports on the carcinogenic properties of *iso*-propylic ether of 3-chloro-phenylcarbamic acid, applied as herbicides in a number of countries. Concern is felt in the United States about the carcinogenic properties of certain organochloric insecticides.

The danger of chemicals to public health does not lie only in the possibility of accidents (acute intoxications). The chief danger to the health of the population lies in the prolonged chronic action of certain preparations inducing pathological states which are not ordinarily diagnosed as intoxication. Considerable data have been presented by American investigators on the rise in the incidence of diseases of the liver and gastro-intestinal tract, and of cardiovascular and nervous diseases among the population of the United States and a number of other countries, as a result of the extensive use of DDT and other organochloric compounds.

The duty of toxicology as the science of the toxic properties of chemical substances is not only to determine the lethal doses of one substance or another, but also to ascertain the possibilities of its chronic effect on the human organism over a prolonged period of intake in small quantities. A preparation which is widespread throughout the world, like DDT, has only a slightly toxic effect on the organism in a single dose, and the probability of poisoning with a lethal outcome is insignificant. When taken into the organism in various ways, however, DDT accumulates in the fatty tissues and is retained in them for a long time. Even in slight quantities, DDT acts very slowly, but destructively, on the organism. The increase in liver disease incidence observed in many countries is to a certain extent due to the intensified use of DDT and other organochloric insecticides.

Investigations conducted in the toxicological laboratory of the Kiev Institute of Labour Hygiene and Occupational Diseases have shown that DDT, chlorindane, hexachlorane, heptachlor and other organochloric preparations, even when taken into the organism in small quantities, accumulate in it and afterwards give rise to various diseases. The investigations of the Ukrainian Institute of Nutrition have shown that, if a plant is treated with DDT during the period of fructification, the preparation is retained for a long time in the fruits. When milch cows are given repeated treatment to combat blood-sucking insects, DDT is eliminated in the milk during a period of over three months in quantities endangering the health of people consuming the milk. DDT does not break down under natural conditions or as a result of cooking. On the basis of the results obtained in these investigations, the State Sanitary Inspection of the U.S.S.R. has adopted an enactment prohibiting the treatment of plants with DDT during the period of fructification and providing for other restrictions.

GAS CHROMATOGRAPHY: DEFINITIONS AND RETENTION PARAMETERS

LEADING workers in gas chromatography soon realized that published retention data would have much greater value if they were reported in a manner which made quantitative comparison possible, and in 1956, at the first committee meeting of the Gas Chromatography Discussion Group of the Institute of Petroleum Hydrocarbon Research Group, a sub-committee was formed to consider recommendations on presentation of data. Although differences between the views of this sub-committee and American workers were settled early in 1958, it was evident that international agreement was desirable, and approaches to the International Union of Pure and Applied Chemistry resulted in the formation of a Task Group to consider the matter. In addition to presentation of data, the Task Group also considered nomenclature, its terms of reference being to encourage the use of established conventions provided they were consistent with accepted theory, to select a single term where several existed, and to eliminate ambiguity of definition. The preliminary report of the Task Group was made available to the Discussion Group early in 1960 and has now been published in *Pure and Applied Chemistry*, the journal of the International Union.

At an informal symposium held at the University of Liverpool on October 21, where the Discussion Group was welcomed by Dr. G. Skirrow, the International Union of Pure and Applied Chemistry recommendations were discussed, and papers on alternative methods of presentation were read. The chair for the morning session was taken by Mr. C. S. G. Phillips and for the afternoon session by Mr. R. P. W. Scott.

After reviewing the historical background and acknowledging a valuable contribution to the work by the Groupement pour l'Avancement des Méthodes Spectrographiques, Dr. D. Ambrose, chairman of the International Union Task Group, discussed some of the points which had been considered in drawing up the recommendations summarized below.

The technique of gas chromatography is divided into gas–liquid and gas–solid, depending on whether separation is effected by partition or adsorption. Definitions are given for the following terms: sample injector, column (solid volume, liquid volume and interstitial volume), detector (differential and integral), carrier gas, liquid phase, solid support and active solid. In gas–liquid chromatography the stationary phase comprises the liquid phase without solid support, and in gas–solid chromatography the stationary phase is the active solid. On the chromatogram, base line, peak, peak base, peak area, peak height, peak width and peak width at half-peak height are defined and illustrated. The Martin and James pressure gradient correction factor is designated j. Uncorrected and corrected (factor j applied) retention volumes measured from the point of injection are respectively V_R and V°_R. The uncorrected retention volume for a non-absorbed gas is termed gas hold-up, V_M. The adjusted retention volume V_R–V_M is designated V'_R and becomes net retention volume V_N when the correction factor j has been applied. These parameters are reported at the column temperature. Specific retention volume V_g is the net retention volume per gram of liquid phase corrected to the volume at 0° C.

Retention data should be published on an absolute basis or relative to a pure solute for which an absolute value is known at the operating temperature, and, wherever possible, the variation with temperature should be reported. Relative retention r of two components is given by:

$$r_{1,2} = \frac{V_{g_1}}{V_{g_2}} = \frac{V_{N_1}}{V_{N_2}} = \frac{V'_{R_1}}{V'_{R_2}} \neq \frac{V_{R_1}}{V_{R_2}}$$

The theoretical plate value n of a column is used to express column performance (previously referred to as column efficiency), and peak resolution is given by twice the difference between retention (in volume, time or distance) divided by the sum of the peak widths in the corresponding units. Sample size, method of injection, and detector dead volume are factors which can affect the values obtained for retention parameters, column performance and resolution unless corrected for. The recommendations are preliminary and the Task Group is to exist for at least another two years; comment and suggestions will be welcomed by Dr. D. Ambrose (National Chemical Laboratory, Teddington, Middlesex, England).

Mr. G. R. Primavesi (Distillers Co., Ltd.), expressing the views of an analyst on the presentation of data, maintained that whatever terms were used they should be relative as well as absolute, and that chart distance took pride of place over time or volume. The theoretical treatment of Martin and James was adequate, although the analyst had no need for the term V°_R. Retardation volume was preferred to net retention volume, and a plea was made that the expression for calculating j be published in a form which could not be misinterpreted. Mr. Primavesi commented on the modes of presentation of specific retentions, and expressed the view that it was more convenient to have retention data measured at the column temperature.

Replying to these and other comments which had been received, Dr. Ambrose stated that, although the relation of partition coefficient to retention data is included in the recommendations, it is not regarded as a retention parameter but merely as an example of one of a number of physical constants which can be determined by gas chromatography. The Task Group had taken as a fundamental experimental quantity the retention volume V_R, which is a volume of gas; the fact that the actual measurement was made in time or distance was irrelevant. The term V^{0}_R was retained with its original definition on historical grounds but with the qualification that "this quantity is of limited use because it is influenced by the volumes of sample injector and detector as well as the interstitial volume of the column", because Martin and James made no mention of the significance of measurement from the air peak and, in fact, defined the ratio V_{R_1}/V_{R_2} as relative retention. Some confusion had arisen over the use of the term V_g; it had been used to describe specific retention per gram at the column temperature and at 0° C. There was very little to choose between the two interpretations, and the latter was chosen for recommendation because flow-meters are usually calibrated at 0° C and the plot of V_g at 0° C. against $1/T$ is linear. Authors are advised to indicate the definition of V_g used when reporting results.

Speaking as a teacher requiring a logically correct approach to theory, Prof. P. Chovin (Laboratoire Municipal, Paris, and chairman of Groupement pour l'Avancement des Méthodes Spectrographiques) preferred to develop it from an ideal column without external dead volume in which retention volume is a 'true' value. This concept does not affect adjusted, absolute and specific values. However, in the case of column performance where retention is measured from the point of injection, comparison of the 'true' and 'real' values gives valuable information on the loss of performance resulting from parts of the apparatus external to the column. The difficulties associated with the measurement of effective injector and detector dead volumes limit the practical interest in 'true' retention parameters. Prof. Chovin also discussed a new parameter, net retention volume per mole of liquid phase, and its application to the determination of activity coefficients. If the activity coefficient of a solvent–solute system at a given temperature was known, the value for the same solute with a different solvent could be determined very simply, and for this purpose a dual column assembly in one vapour jacket had been used.

In a paper by Mr. R. L. Martin (Standard Oil Co., of Indiana), read by Dr. A. Goldup, work was described which was interpreted as indicating adsorption effects at the liquid–gas interface. Such a phenomenon seriously affects the derivation of partition coefficients and other derived thermodynamic functions unless corrected for. In some circumstances the correction could amount to more than 80 per cent of the measured retention volume. With packed columns it had been found that the order of elution of hydrocarbon solutes varied with the amount of liquid phase, and the plot of retention volume per gram of column-packing versus liquid-phase percentage did not extrapolate to zero retention at zero liquid phase. With non-polar liquid phases the deviations were small and were attributable to adsorption on the support. With polar liquid phases the deviations were considerably larger and varied with the liquid phase. Using values for liquid surface area obtained by subjecting the coated support to the gas chromatographic continuous-flow method for surface area of solids, retention volumes were accounted for as the sum of a contribution by solution in the liquid (partition coefficient × volume of liquid) and a contribution by adsorption on the surface of the liquid (adsorption coefficient × surface area of liquid). The contribution by solution increased linearly with increase of liquid phase, but the contribution by adsorption decreased due to the decrease in liquid surface area as the percentage of liquid phase increased. Calculated 'adsorbabilities' for saturated hydrocarbons on polar liquid phases bore an inverse relation to the surface tension of the hydrocarbons. It was also noted that column performance was considerably greater with liquids on which adsorption occurred.

Mr. W. T. Swanton (British Petroleum Co.) questioned the validity of some of the arguments, and indicated that effects due to the solid support, uncoated support in particular, should be considered carefully. The relative values obtained for some of the 'adsorbabilities' were also queried; static experiments and application of the Gibbs adsorption equation might clarify the position. Further discussion on this interesting paper was limited by the absence of the author.

Dr. E. Kovats and Dr. J. F. Smith each presented methods for reporting data which go a long way towards solving the difficulties of analysts, who so often find that their extensive collection of relative values is of limited application because it lacks a common denominator relating the different standards which have been used to cover liquid phases of diverse type and a variety of temperatures.

In the Retention Index Scheme proposed by Dr. Kovats (Laboratory for Organic Chemistry, Zurich) the retention of a solute is given as a value obtained by logarithmic interpolation between the values for two even-numbered *n*-alkanes which, for convenience, are assigned values equivalent to one hundred times their carbon number. The good repeatability and reproducibility of the method were illustrated with the aid of values calculated from retention data published by other workers; however, as with other forms of retention data, possible effects of solid support must be considered and care taken in standardizing on a reproducible support. Except for small molecules such as methanol and nitroethane, where the functional group dominates, the variation of retention index with column temperature is small and linear—about four units per ten degrees centigrade. From the results of a large number of determinations, the difference between the retention indexes of two close-boiling isomers is deduced as five times the difference between their boiling points (°C.). Retention indexes increase by one hundred units on both polar and non-polar phases for the higher members of any homologous series with the insertion of a $-CH_2$ group. Differences in values obtained on polar and non-polar phases can be ascribed to solute type. For non-polar hydrocarbons the difference is zero. Dr. Kovats presented evidence that bonding types and functional groups in a molecule can each be assigned a difference value, which for a given polar liquid phase are additive.

With Dr. Smith's theoretical nonane or R_{x9} method, which has been used extensively and proved invaluable in the laboratories of the Natural Rubber Producers' Research Association, all values are expressed relative to the retention time for *n*-nonane, which is obtained by extrapolation or interpolation from the logarithmic retention time versus carbon number plots with *n*-alkanes. A simple relation between the R_{x9} value and the retention index was shown to exist. Statistical examination of the results obtained for various chemical classes on squalane and dinonyl phthalate at several temperatures showed that the repeatability for the whole calibration procedure and determination of relative value were at least as good as for methods involving the use of one or more standards within the chromatogram.

C. G. SCOTT

CONDITIONING AND ELECTRO-PHYSIOLOGY

A PAVLOVIAN Conference on "Higher Nervous Activity" was held during October 13–15 in New York, under the auspices of the New York Academy of Sciences and the Academy of Medical Sciences of the U.S.S.R. This Conference follows, in its main lines, a number of recent international meetings such as the Moscow Colloquium on "Electroencephalography and Higher Nervous Activity", the Montevideo Symposium on "Brain Mechanisms and Learning", and the Macy Conference series on the "Central Nervous System and Behaviour". These Conferences have been promoted because of widespread belief, explicitly stated by Magoun in his contribution to the New York Conference, that "the most striking recent progress in the biology of Higher Neural Activity has unquestionably come from electrophysiological analyses of the mechanism of learned behaviour. Knowledge has especially been advanced by marriage between the electrical recording techniques of Western neurophysiology and the classical Pavlovian approaches to the study of conditioned reflexes cultivated so extensively in the U.S.S.R.". The proceedings of the entire meetings will be published in the *Annals of the New York Academy of Sciences*. Attendance at the meetings averaged about eight hundred persons on each of the three days.

How does this Conference conform to a reformulation of Parkinson's law, namely, that regardless of new material available the number of words spoken at a conference will expand to fill the *Proceedings* published a year or two later? On the whole, expectations are fulfilled. There are some semi-philosophical discussions "on the relationship between neurophysiology, psychophysiology, psychopharmacology and other disciplines", which are not likely to throw much light on the difficult task of analysing the cortical and subcortical events taking place during conditioning. We have a rather expanded account of "The Role of Slow Waves in Differential Inhibition" based on records obtained from electrodes implanted with unknown accuracy "in the visual cortex, auditory cortex, medial suprasylvian cortex, dorsal hippocampus, ventral hippocampus, mesencephalic reticular formation, centralis lateralis, centre median, medialis dorsalis, and the lateral geniculate body" of two cats in which the conditioned avoidance response to flickering light was established. The results of this "admittedly crude and exploratory pilot study" are not particularly informative; conclusions are of the nature that "our electrographic data indicate that cortical slow waves are associated with inhibition, although they do not preclude excitation"; and we also have "The First Law of Bio-Cybernetics: Unidirectional Rate Sensitivity", which attempts a mathematical expression of heart-rate and pupillary reflexes. This paper will, no doubt, appeal to mathematicians familiar with Laplace operators, but will leave most biologists doubtful about the applicability of this type of analysis to sets of data still very much in search of appropriate causal factors.

From the point of view of the Western reader, no doubt the most interesting and important papers will be the Russian ones, particularly those by P. K. Anokhin and E. A. Asratyan. These not only give a summary of work done over the years in the laboratories of these two contributors but also report work which, to the present reviewer at least, was unknown and interesting.

Anokhin, after directing attention to the fact that many years ago Pavlow had already emphasized the importance of subcortical centres in ensuring a "high energy level for the functional interactions among the cortical elements", began his talk "with a formulation of some perfectly obvious contradictions which are now arising between the basic ideas of modern instrumental neurophysiology and the physiology of higher nervous activity. . . . The main contradiction is in the understanding of the essence of the ascending non-specific activity effect of the reticular formations of the stem and thalamus on the cerebral cortex". The problem, as it appears to Anokhin, is this: "The generally accepted interpretation of this phenomenon assumes that this effect is generalized throughout the entire cortex and probably all the cortical cellular elements. *Desynchronization*, that is, transformation of the slow-wave and high-amplitude electric activity into low-amplitude and high-frequency electric activity, is usually regarded as electrophysiological expression of this generalized activation. . . It is silently assumed that this activity concerns *all the cell elements of the cortex*, hence, its designation as 'diffuse' activity".

This diffuse and non-specific activity contrasts with the very highly specific formation of conditioned reflexes, that is, the creation of "the temporary bond between the given stimulus and any inborn activity of the organism". As Anokhin puts his problem: "How can generalized and apparently diffuse activation of the cerebral cortex be combined with the finest selective interactions between the cortical elements down to and including individual synaptic contacts?" Anokhin's very lengthy address summarizes the extensive work of his school relating to this problem, which has proceeded along two main lines. In the first place he has made a comparative evaluation of the activating effect of the cerebral cortex under conditions of two biologically opposed states of the animal—defensive and alimentary; and secondly, he has worked by means of stereotaxic placement and micro-electrode techniques of evoked potential. He studied "the ways of the ascending influences on the cortical element under different pharmacological effects and at different stages of ontogenetic development". The results of all this work are interpreted by him as throwing considerable doubt on the notion of 'diffuseness' as applied to the activating effects of the subcortical structures.

Asratyan is concerned with "The Question of the Initiation and Localization of Various Types of Cortical Inhibition in the Elements of the Conditioned Reflex Arc". He opposes both the two traditional views, that is, "that conditioned inhibition is initiated in the cortical nerve cells of the focus of the conditioned stimulus", as well as the alternative view, "that conditioned inhibition is initiated in the nerve cells of the focus of the unconditioned stimulus". (He also rejects the view of Kupalov, which combines these two hypotheses.) Asratyan's own theory amounts to saying "that conditioned inhibition is initiated primarily in the elements of the conditioned connexion itself, which we represent as a chain of internuncial neurones". Most of the work he quotes in support comes from experiments on "transswitching"; this term is used by him to designate an experimental paradigm in which ". . . a single indifferent stimulus is given two different signal values at the same time. This is done by reinforcing the stimulus differently in two different experimental conditions or situations". These experiments are interesting, but scarcely conclusive; Asratyan does not, for example, discuss at all the difficulties which would be presented for his theory by the results of Culler's classical experiment of 1938.

It is noteworthy that, while the American and Russian participants at this Conference do occasionally take notice of each other's work, yet in the main there is no real bridge from one group of workers to the other. This may be a question of nationality and politics; but other possibilities spring to mind. Electro-physiologists in the United States, and in Great Britain too, pay just as little attention to the work, or the contribution, of Western psychologists active in the field of modern learning theory and conditioning as they do to the Russians; the encapsulation of separate disciplines is more likely to be the villain of the piece, rather than national or political prejudices.

H. J. EYSENCK

THE CHANGING INDUSTRIAL ENVIRONMENT

THE theme of the annual conference of the Institute of Personnel Management which was held at Harrogate during October 7–9 was "People and Machines".

Various developments in personnel management and industrial relations were considered by the 1,200 individuals who attended from industrial companies, local and central government departments, universities and other higher educational institutions throughout the world. The tone and conduct of the conference were again on such a high plane that it is bound to have far-reaching consequences. Responsibility for organizing a conference which, outside those of political parties and trade unions, is probably the biggest in Great Britain lies mainly with Miss H. E. Cutting, who must be congratulated for the quietly efficient way in which these conferences take place.

The opening address was given by the newly appointed Minister of Labour, the Rt. Hon. John Hare, who, after reviewing the general principles on which the Government acts in the field of industrial relations, described the measure of success which stems from the British system of free negotiation. In the twelve months from June 1959 to June 1960, for example, more than 300 important claims for increased wages or shorter hours covering practically the whole of industry were settled peacefully, while less than ten resulted in disputes which led to strikes.

Strikes can be put broadly into two categories. First, there are strikes over clear-cut economic issues affecting wages or conditions of work. These, if they take place after the procedure of negotiation has been followed and failed to produce agreement, are one of the consequences to be accepted as inevitable from time to time in a free society where those who work have the right and power to seek the conditions of employment they feel that they are entitled to.

The second category contains the large number of strikes in individual establishments over a wide variety of difficulties, not only wages, which arise between management and labour or between different groups of workers. They arise over such issues as discipline, dismissals, redundancy, working arrangements and union membership. The causes of many of them are sometimes obscure—at least to the general public. Most of them are unofficial.

This second category of strike has recently given rise to public criticism. Industry itself, both employers and trade unions, is gravely concerned about them. Mr. Hare indicated that these criticisms and fears are justified because these strikes indicate continuing weakness in the present system. The Minister recommended that the two sides of the motor-car manufacturing industry should set up a top-level study group, or informal committee, to produce constructive proposals for overcoming the problems which cause regrettable stoppages within the industry—whether these problems arise on the management or the workers' side. The Minister indicated his readiness to help such a group.

At one of the sectional meetings the issue of whether collective bargaining should be carried out nationally or locally was examined. Prof. E. H. Phelps Brown showed how, in the years of full employment since the Second World War, there has been a substantial increase in the extent to which actual terms and conditions of employment are regulated by bargaining between firms and their own workpeople. Much of our collective bargaining now has a two-tier structure: from time to time each employee gets a rise the amount of which is fixed by an industry-wide bargain, but this actual rate depends also on a bargain struck within the firm. The procedure by which these local agreements are reached vary widely, and many are informal, but, in one way or another, they involve negotiation between a firm's management and its own work-people.

Is this development to be welcomed or resisted? Negotiation firm by firm avoids the danger of industry-wide bargaining and that rises in money wages will be granted too readily, because all competitors will have to pay them and so expect to be able to cover them by a general rise in selling prices. On the other hand, there is a danger that the different rates for the same job in different firms will give rise to unrest and whip-sawing.

Mr. G. Moxon, of United Glass, Ltd., was firmly against any proposal to decentralize wage-rate bargaining. Such a proposal presents a major change to the established and traditional system affecting all companies in some degree or other, but affecting most markedly multiple companies with factories in different parts of the country.

Moxon's objections to the proposal may be summarized as follows: there is no evidence that the decentralization of wage negotiations would result in more acceptable bargains than is the case under the present system. On the contrary, it is at least possible that disputes would increase and factory relations would become worse if wage-rate bargaining were brought to the factory floor. There is no evidence that the decentralization of negotiations would diminish such tendencies as there are to a wage–price spiral; decentralized wage-rate bargaining is likely to have the effect of transferring a good deal of responsibility for bargaining away from the senior ranks of trade union officials (for example, national officers), into the hands of less-experienced, and sometimes less-successful, officials at plant-level where shop stewards will exercise a considerable influence; factory bargaining for increases beyond minima would necessitate some unified body of unions to negotiate on a common front with company management. This would mean a unified body embracing craft and general workers' unions. Without this safeguard, management would be exposed to leap-frog tactics by the various unions with which it negotiates.

The education and training of technicians were elaborated by Mr. J. S. Newton of Pilkington Bros., who used sections of the Crowther Report to distinguish between the craftsman, technician and technologist. The former has sufficient skill to make components, the second has enough training and industrial experience to supervise the carrying out of various skills, and the technologist has sufficient training to apply highly complicated scientific and technological methods to production. The technician has been brought into existence in order to solve a two-fold problem: (*a*) craftsmen in modern times are being asked to tackle work beyond their scope; (*b*) highly trained men are being wasted on work requiring much less potential. The technician must have extensive grounding in many skills, and must be intellectually more able than the craftsman. He must supply the 'know-how', and acquire the arts of communication and management. He forms the vital link between the other two grades

Industry must decide what its requirements are in each grade, and begin by careful selection for each grade, considering educational qualifications along with general background. This may help to decrease the failure-rates between commencing and finishing a college course.

Training given at work must be carefully planned, and watched closely if it is decentralized; trainees should not be used as cheap labour. The training officer must have a clear-cut policy to implement, and planning and reports must be centralized. If training is centralized, it must not be isolated from production. If it is decentralized it must be on a systematic basis, with set periods under different operator/trainers, who must be paid special rates so that their earnings do not suffer. Industrial training must supply the trainee with as wide a field of experience as possible, filling in gaps with short-period visits to other factories, on an exchange system. Trainee technicians must have early experience of dealing with emergencies. Training must be devised to develop and mature their personalities.

The British contribution to the development of personnel management in newly industrializing countries was discussed by two speakers with considerable experience overseas. Mr. F. J. Pedler, of the United Africa Co. Ltd., declared that the golden rule for staff relations is to treat African workers in Africa as one would treat English workers in England. "If one begins by treating an African trade union as one would treat a respectable trade union in England one cannot go very wrong." The assumption is too commonly made that trade unions in Africa are subversive, communist, corrupt and unrepresentative. Some African trade unions are fully responsible. It is true that some are dominated by communists, and others, without being communist, are identified with political parties in their own country. The influence of Israel on trade union structure has been strong in some African countries.

Employers should be bold and sympathetic in recognizing unions and helping them to develop along the right lines. The technique of a joint industrial committee, for example, has been widely used in Africa with success.

The basic fact about African labour is that it is very scarce. It has to be managed as a scarce commodity, as it already commands the price of a scarce commodity: the position in Africa is very different from India.

It is important that political urges and economic development should be brought into harmony and made to serve useful ends. In this problem, personnel management has a big part to play. Such management must handle the basic problem of labour scarcity in such a way that the cost of wages and fringe benefits do not put a brake on investment.

The training of staff at all levels is essential. One of the important things which England can pass on to Africa through good personnel management is the conception of a commercial company as a community of men united for a common purpose: and, within the larger community, the conception of each unit—whether it be a department or a training school —as a team in which there are corporate interests. The most important step in achieving such a spirit is to promote Africans to be managers and to associate them fully in running the business.

Mr. John Marsh, Industrial Welfare Society, suggested that, in helping the development of personnel management in countries overseas, the British contribution should insist that our daily practice in industry and commerce should be an example. This is particularly true if the British control or administer expatriate enterprises in foreign lands.

Personnel officers and other managers who serve abroad for a portion of their lives are especially important as ambassadors of modern Britain. The Government is making a special effort to ensure that teachers going to the Commonwealth should have safeguards in their future careers, and Civil servants serving in other lands are now more satisfactorily protected. Service in industry and commerce overseas is still a handicap instead of a credit to a man's career. By sharing our knowledge and experience with indigenous managers and specialists sent to the United Kingdom for training and development, Britain makes a considerable contribution to the development of good industrial relations overseas.

The closing address was given by Sir Ben Lockspeiser, of Tube Investments, Ltd., who described the evolution of machines manufactured by man to create the environment in which he now lives. But, although man is good at designing machines, he is not so good at understanding human behaviour, and the introduction of electronic machines could have lamentable social consequences if the personal problems involved are neglected. Many people are bound to be affected personally. Considerable changes of technique cannot be brought about overnight. The period of adjustment is long enough to give those in authority the opportunity of using the means at their disposal, such as retraining schemes and the adaptation of recruitment and wastage-rates, to anticipate consequences and work out the necessary human measures to meet new circumstances. Good personnel management must accompany a developing technology.

T. H. Hawkins

EPIDEMIOLOGY OF BLACK RUST OF WHEAT

A SYMPOSIUM on "Epidemics of Black (Stem) Rust of Wheat" was held in the afternoon of October 7 under the auspices of the British Mycological Society. Mr. L. Ogilvie (National Agricultural Advisory Service, Bristol) said that the alternate host of black rust, wild barberry, does not appear to be implicated in the origin of uniformly distributed and almost simultaneous epidemics in the south-west of England. It was therefore decided to find whether uredospores are transported from the Continent in the same way as they are carried northwards from Texas into the American wheat belt.

Collaborative investigations which have been proceeding in Western Europe and North Africa since 1958 show that the disease overwinters in the uredospore stage on wheat and grasses in North Africa and Southern Iberia, and spreads northwards in summer. Observations on field crops and on some thirty widely scattered 'rust nurseries' (small plots of varieties of spring wheats) were organized from Bristol. About a fortnight after uredospores were trapped in quantity, rust was found in the fields and from then on became common in most of the southern coastal areas, the Channel Islands and Isles of Scilly and, later, to a lesser degree, in the whole of the southern half of England.

Dr. I. G. Thorpe (National Agricultural Advisory Service, Bristol) stated that the oat, barley, and some grass varieties of *Puccinia graminis* are not uncommon on barberries in England, but the wheat variety has not been found in recent years. The simultaneous appearance of the disease over a large area suggests a widespread inoculum. Uredospore deposits on spore traps can usually be correlated with air trajectories from countries to the south, as is explained later. Up to the present, the spectrum of rust races recognized in England greatly resembles that of the Iberian Peninsula and France. Mr. H. A. Hyde and Mrs. K. F. Adams (National Museum of Wales, Cardiff) reported that black rust uredospores have been found from May onwards in the spore catches organized by the Asthma and Allergy Research Unit at Cardiff and the Bishop Rock Lighthouse, often before wheat is affected in England. Simultaneous catches of black rust spores have often been made at points more than 150 miles apart. The theory of spore clouds originating from the Continent is supported also by catches in spring of pine pollen, which apparently came from southern Europe.

Mr. W. H. Hogg (Meteorological Office, Bristol) described how air trajectories could be prepared from weather maps, and gave estimates of the errors involved. Uredospore catches in London during the period July 4–10, 1959, were accompanied by air trajectories from the Continent. When the air came from other quarters, spores were few or lacking. Dr. J. M. Hirst (Rothamsted) dealt with theories underlying the liberation, transport and deposition of rust uredospores. Sampling from aircraft traversing southerly airstreams over the English Channel have shown large numbers of spores at heights up to 7,000 ft.

Conclusions which may be drawn from the symposium are that a severe black rust epidemic in England depends on susceptible varieties of wheat (all varieties grown here are susceptible to the common West European races of black rust), lateness of the wheat crops, transport of spore clouds arising from epidemics to the south by one or more favourable wind trajectories in late June or early July, and warm and humid weather at the time of infection and for several weeks later.

TROPICAL ECOLOGY

ECOLOGICAL terminology and concepts applicable to, and developed under, temperate conditions sometimes have little relevance to the vegetation of the tropics, and a journal devoted especially to tropical ecology will be welcomed by many as meeting a real need, so long as it does not encourage ecologists in differing climatic zones to overlook the essential unity of their subject.

Nos. 1 and 2 of Vol. 1 of the *Bulletin of the International Society for Tropical Ecology* give little indication of the extent to which this new journal will, in fact, meet the need which undoubtedly exists. No. 1 contains a presidential address: "Why Tropical Ecology?" by J. C. Sen Gupta, as well as abstracts of 22 papers, mainly on tropical forests, given at a symposium on forest ecology held at Bombay. In No. 2 are rather longer accounts of 21 papers read at a forest ecosystem symposium which was a part of the ninth International Botanical Congress held at Montreal. There is thus an absence of research papers of the conventional type; but it is useful to have the views of experts from many countries on forest classification assembled together in a single volume in this way.

No indication is given as to whether succeeding numbers of this journal will be restricted to abstracts of papers read at symposia and conferences, or whether the journal is to restrict itself to tropical plant ecology. Until it is possible to see what permanent form this new journal takes, it can be given only a qualified welcome, although a forum in which ecologists of the tropics can discuss their special problems and report on their work is undoubtedly needed.

The *Bulletin* is edited by Dr. G. S. Puri, general secretary of the Society, 10 Chatham Lines, Allahabad, India, assisted by an editorial board with representatives from Japan, India and Great Britain. It is not priced, but is distributed to members of the Society (annual subscription 15 rupees, or £1 2*s*. 6*d*.).

OBSERVATIONS OF FALL-OUT IN INDIA DURING THE PERIOD OF CESSATION OF NUCLEAR TESTS

By K. G. VOHRA and V. S. BHATNAGAR

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THE recent measurements of fall-out in different parts of the world have provided evidence of a shorter time of residence of fission products in the stratosphere than had been estimated earlier[1-5]. Martell[1,2] has suggested residence-time in the stratosphere of 6 months–1 year for the tests carried out in the polar regions and 3–5 years for the tests carried out in the equatorial regions. The investigations on fall-out carried out in Norway[3] have shown that the airborne activity at the ground-level starts increasing shortly after high-yield tests, reaches a peak value in about six to seven months and then decreases with a half-life of about one month. These measurements also suggest a short value of mean residence-time in the stratosphere.

The ground-level airborne fall-out activity in India[6] after October 1958 showed a trend somewhat similar to that observed in Norway. A rise in airborne activity was observed soon after the completion of high-yield tests carried out in the polar regions in October 1958. This increase continued, and a maximum was reached at all the stations after about four months, that is, during February–March 1959. This activity decreased with a half-life of about 40 days, suggesting residence-time in the atmosphere of about 60 days.

We have also studied the ratio of zirconium-95 and cæsium-137 activities in samples of rain-water from Srinagar and Bombay during the period December 1958–September 1959. The change in this ratio with time seems to provide some information regarding the relative proportions of fall-out debris from the old tests and the October 1958 polar tests. The mean residence-time in the atmosphere has also been estimated indirectly from this ratio and is found to be about 60 days.

The obvious consequence of short residence-time in the stratosphere is an increase in the external gamma-radiation dose for several months after high-yield tests. The radiation dose from the gamma-emitting isotopes (zirconium-95 + niobium-95), (cerium-144 + praseodymium-144), ruthenium-106 and cæsium-137 after the October 1958 test series has been estimated from the observed levels of activity.

Airborne Radioactivity

Fig. 1 gives the concentrations of radioactive fall-out fission products in the air at ground-level at different stations in India for the period October 1958–June 1959. The airborne radioactivity shows a steady increase after October 1958, reaching a peak value in about four months. The peak in activity is observed at all the stations, except Ootacamund, during the months of February and March, after which the activity decreases with a half-life of about one month.

The increase in activity to the peak value during the period of about four months after the October 1958 test series may be explained as due to the storage of the debris in the troposphere determined by the mean residence-time in the troposphere, which is of the order of 20–30 days. The decrease in airborne activity at ground-level will be governed by the trend of decrease in the troposphere, which in turn would depend on the residence-time in the stratosphere.

The levels of airborne radioactivity averaged for all the stations for each month and corrected for radioactive decay to October 15, 1958, have also been plotted in Fig. 1, and a smooth graph has been drawn through these points to give a picture of the general trend of variations of airborne activity at ground-level. This graph shows that, after the peak is reached, the activity decreases with a half-life of 40 days, as determined from the slope, corresponding to a mean residence-time in the atmosphere of about 60 days.

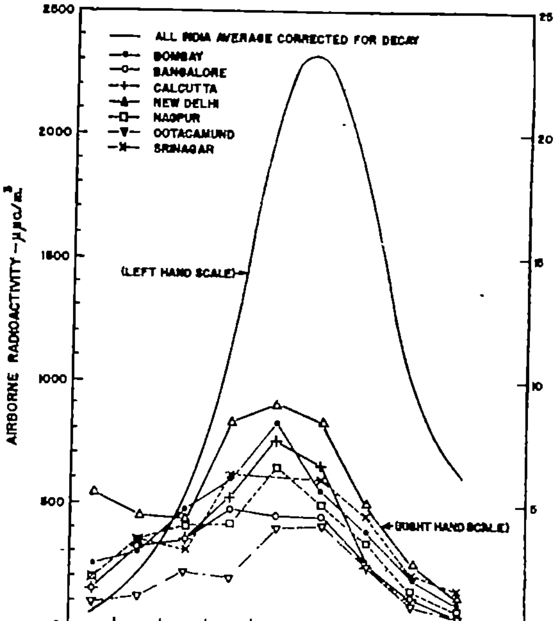

Fig. 1. Average monthly levels of airborne radioactivity at different stations in India during October 1958–June 1959. The smooth curve shows the level of airborne radioactivity averaged for all the stations for each month and corrected for radioactive decay to October 15, 1958

Table 1. INFINITY EXTERNAL GAMMA-RADIATION DOSAGE FROM THE MONTHLY DEPOSITION ZR-95 NB-95, CE-144 PR-144, RU-106 AND CS-137 AT SRINAGAR DURING OCTOBER 1958–JUNE 1959

Month	Zirconium-95 + niobium-95 (m.rems)	Cæsium-144 + praseodymium-144 (m.rems)	Ruthenium-106 (m.rems)	Cæsium-137 (m.rems)	Total (m.rems)
October	0·14	0·039	0·048	0·76	0·99
November	0·22	0·048	0·026	0·81	2·11
December	1·69	0·180	0·306	3·08	5·25
January	1·85	0·178	0·318	3·04	5·39
February	1·03	0·169	0·542	5·62	7·36
March	2·79	0·256	0·607	6·53	10·18
April	1·22	0·169	0·860	5·25	7·50
May	1·55	0·240	0·739	7·40	9·93
June	0·14	0·048	0·140	1·63	1·96

Zirconium-95/Cæsium-137 Ratio in Rain-water

Monthly collections of rain-water from Srinagar for the period October 1958–June 1959 and individual rain samples collected at Bombay during June 1959–September 1959 were analysed for zirconium-95 and cæsium-137 activity using a gamma-ray scintillation spectrometer. The scintillation spectrometer consisted of a 10-channel pulse-height analyser, and a well-type sodium iodide scintillation detector. Each sample was counted several times over a period of 5–6 months, and the 0·75-MeV. gamma-ray peak due to zirconium-95 verified for its 65 days half-life. The activity of each sample was corrected for decay to the middle of the collection period.

Fig. 2 shows the zirconium-95/cæsium-137 ratio for the period October 1958–September 1959 for both Srinagar and Bombay samples. It will be seen that the ratio rises at Srinagar in December 1958. This indicates the increase of fresh nuclear debris in the troposphere, presumably from the October 1958 tests. During December 1958–May 1959 the ratio decreases, and the points lie close to the decay curve of zirconium-95 (half-life 65 days) shown by line *AA*. The observed ratios for June 1959 and subsequent samples show an increasing departure from line *AA*. The departure may be explained as follows.

As will be seen from Fig. 1, the airborne activity which had shown an increase following the October 1958 tests came down to a fairly low level in June 1959. After June 1959 one would therefore expect an increasing proportion of fission products from the earlier equatorial tests, which probably have a larger residence-period in the stratosphere. Thus the ratio zirconium-95/cæsium-137 in the precipitation samples after June would be more dependent on the relative abundance of cæsium-137 from equatorial and polar tests. If we assume that the fall-out from the polar tests in 1958 is superimposed on a constant background of cæsium-137 from earlier tests, and that all the zirconium-95 activity in the atmosphere during June–September 1959 is from October 1958 tests, the departure of zirconium-95/cæsium-137 ratio from the calculated value from June onwards should be due to the rapid depletion of the debris of polar tests in the atmosphere. The residence-time in the atmosphere for the debris from polar tests is estimated from a least-square fit to the points between June and September as shown by line *BB*. The slope of this line gives $(\lambda_R + \lambda_T)$, where λ_R is the radioactive decay constant for zirconium-95 and $\lambda_T = 1/\tau$, where τ is the mean residence-time in the atmosphere for debris from polar tests. The value of τ is calculated from line *BB* to be about 60 days.

Estimates of the External Gamma-radiation Dose

The levels of deposition of (zirconium-95 + niobium-95), (cerium-144 + praseodymium), ruthenium-

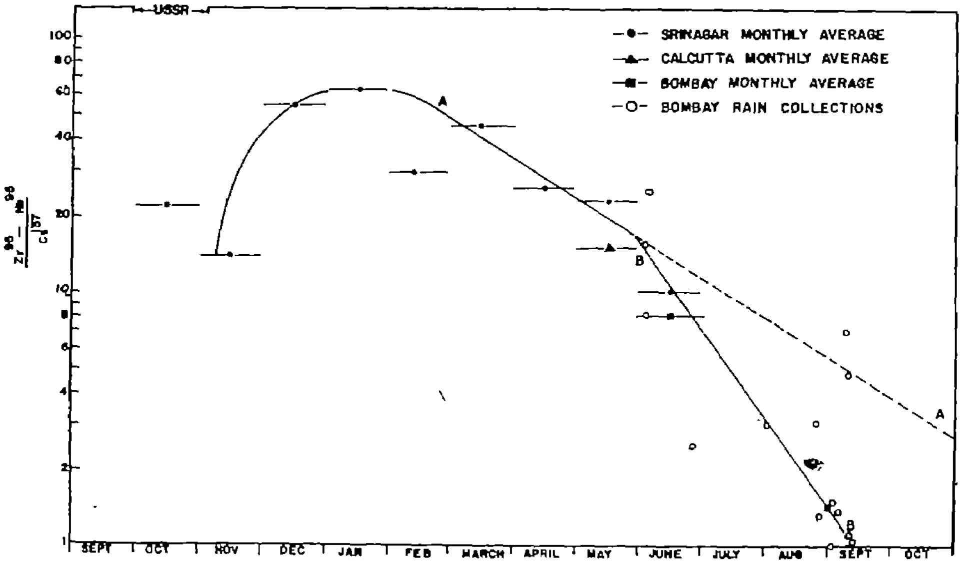

Fig. 2. (Zirconium-95 + niobium-95)/cæsium-137 ratio in rain-water for the period October 1958–September 1959. Line *AA* corresponds to the radioactive decay of zirconium-95, and line *BB* is a least-square fit to the experimental points during June–September 1959

106 and cæsium-137 were measured in the monthly collections of rain-water at Srinagar for the period October 1958–September 1959. The infinity gamma-dose from these isotopes has been estimated for the different months and the results are shown in Table 1. The maximum dose is about 10 m.rems observed in the month of March 1959. The total dose from these isotopes during October 1958–June 1959 is about 50 m.rems. The actual dose will be higher than this, because the contribution from the shorter-lived isotopes has not been considered.

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MEMORY EFFECT IN THE THERMAL INITIATION OF EXPLOSIVES

By Dr. WERNER R. HESS and Dr. RUFUS C. LING*

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THE 'memory effect' is a quite well-known phenomenon in the thermal sensitization of explosives. It manifests itself through the apparent equality between the time of ignition delay (induction period) of an unsensitized sample and the sum of the durations resulting from several thermal treatments. Thus, Ubbelohde[1] found that the total induction periods of lead azide and mercury fulminate heated at a given temperature T_0 are practically the same whether measured in one or more stages. Multi-stage heating involves heating a sample at T_0 for a time t_1 followed by chilling and repeating this cycle one or more times until an explosion is finally observed. Algebraically, this can be stated by the equation:

$$\tau_0 = t_1 + t_2 + \ldots + \tau \qquad (1)$$

where τ_0 is the one-stage induction period, $t_1, t_2 \ldots$ are the durations of the first, second, etc., heating stages, and τ is the time to explosion associated with the last heating stage. It is experimentally observed that τ_0 is a lower limit for the sum of terms on the right side of equation (1).

Jones and Jackson[2] observed the memory effect in both primary and secondary explosives. These authors presumed this effect to be related to the kinetics of the decomposition reaction, since a shortening of the induction period occurred only where autocatalysis by condensed decomposition products had been definitely established. In this article an attempt is made to explain the effect in terms of equation (1) and a particular autocatalytic reaction equation. Then, the memory effect is seen to depend primarily on the concentration of catalyst formed during the thermal treatment, since the rate of the decomposition reaction naturally increases with increasing initial concentration of catalyst.

Measurements of the induction period as a function of temperature generally follow the Semenov equation:

$$\ln \tau = B + \frac{E}{RT} \qquad (2)$$

which permits one to calculate values for an apparent activation energy E. Theoretical justifications of the validity of equation (2) have been supplied by several authors. For example, from the fundamental thermal theory of explosion by Franck-Kamenetski[3] (which was extended to solids by Rideal and Robertson[4]), Cook[5] derived a relation of the Semenov-type which takes into account both the heat conduction and the geometry of the sample. On the other hand, Eyring and co-workers[6] gave an expression for the induction period by assuming a first-order reaction and neglecting losses of heat by conduction. Then again, Semenov[7] treated self-ignition of gases on the basis of an autocatalytic reaction equation and the influence of heat conduction. Similarly, Andreev[8] showed that a Semenov-type relation for the induction period is obtained when an autocatalytic reaction equation is used as will be discussed later.

The basic problem in any theoretical explanation of the induction period derives from the difficulty of finding a kinetic law which is valid for the reaction under consideration. In the chain theory[9] possible mechanisms for reactions in solids have been described to a large extent. However, in spite of extensive agreement with experiments, the general validity of this theory has not yet been established.

Accordingly, we shall assume the simple autocatalytic reaction:

$$B \xrightarrow{A} A + C \qquad (3)$$

which is governed by the kinetic equation:

$$\frac{dx}{dt} = K_0(a + x)(b - x) \qquad (4)$$

Here, x is the concentration of the reaction product A with its initial concentration a, b is the initial concentration of the substance B, and K_0 is the temperature-dependent reaction-rate constant. Equation (4) enables one to derive the relationship between the amount of catalyst x produced after the time t.

It is known that equation (4) is primarily valid for certain reactions in the gaseous phase or in solutions. However, a number of experimental investigations showed that the simple autocatalytic relation is a sound approximation also in cases concerning condensed systems.

Hinshelwood[10] reported that the decomposition behaviour of molten tetryl satisfies equation (4). Prout and Tompkins[11] described the decomposition of potassium permanganate by an expression similar to the solution of the above autocatalytic law (4) derived from the expanded chain theory. The same relation fitted the decomposition curves of some organic compounds as found by Vaughan and Phillips[12]. In the theory of branching and terminated chains the following equation is derived[13]:

$$\frac{dx}{dt} = K.x.(x_f - x) \qquad (5)$$

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which neglects chain interference at $t = 0$, and differs from the autocatalytic law (4) only by the omission of the initial concentration a. This formal identity emphasizes a similarity of the picture of the Brownian movement and collisions in gases to the branching of chains in solids.

It is, however, not necessary that the kinetic law be fulfilled over the total decomposition-range. An agreement is demanded only up to the accelerating region, where slow decomposition changes to explosion. In the beginning of the reaction, the formation of catalyst nuclei takes place and secures a proportionality to the concentration. At a later stage, these nuclei may grow during the reaction, so that a certain amount of catalyst is screened out from further participation in the catalysis. As the reaction proceeds further, a transition to an interface catalysis will occur. When the interface process becomes dominant compared with the 'linear' chain catalysis the diminution of active catalyst may be described approximately by means of exponents :

$$\frac{dx}{dt} = K_0(a + x)^n . (b - x)^m \qquad (6)$$

An equation of this kind has been used already for complicated gas reactions by Semenov[14].

The simple type of reaction equation (4) will, however, be used for mathematical treatment in this work. That means that a duration of the reaction is considered in which the growth of nuclei is negligible compared to the formation of new nuclei. This is justified very well by the chain theory itself, which claims that the reaction in a solid goes on along certain preferred paths. On the other hand, however, this supposition does not affect the autocatalytic view of the reaction. It seems reasonable that decomposition products may catalyse much more easily those neighbours in the lattice which are already in a higher energy stage. (Edge sites are preferred to surface, etc.) An autocatalytic process demands that an initial concentration of catalyst be present to start the reaction. Mathematically, this is reflected in the integrability of equation (4) from $x = 0$, when $a > 0$ is taken. The concentration a may be represented by lattice defects, etc., and will in general depend on the history of the sample. This explanation does not seem sufficient, however, when a assumes values too large relative to the concentration b as shown later. In these cases, one may follow the suggestion of Andreev[15] by superposing a first-order reaction which is responsible for starting the decomposition :

$$\frac{dx}{dt} = k_1 . (b - x) + k_2 . x . (b - x) \qquad (7)$$

It can easily be seen that equation (7) is identical to equation (4), when a is set equal to k_1/k_2.

In all the following calculations, heat conduction losses will be neglected. First, the limiting case is that in which the temperature is considered to be constant. Then, the existence of a true memory effect according to equation (1) can be shown easily. The isothermal solution (K_0 = constant) of equation (4) permits determination of the time t after which any desired amount x of catalyst has been formed :

$$t = \frac{1}{(a + b)K_0} \ln\frac{b(a + x)}{a(b - x)} \qquad (8)$$

The induction period is assumed to be the time at which the maximum reaction velocity is reached, and is determined for the untreated sample as :

$$\tau_0 = \frac{1}{(a + b)K_0} \ln\frac{b}{a} \qquad (9)$$

and for the preheated sample containing an amount of catalyst $(a + x)$ as :

$$\tau = \frac{1}{(a + b)K_0} \ln\frac{b - x}{a + x} \qquad (10)$$

Addition of equations (8) and (9) and comparison with equation (10) proves the existence of equation (1).

The experimental results show, however, that a true memory effect is never exactly achieved and that only the following inequality :

$$\frac{\tau}{\tau_0} > 1 - t \qquad (11)$$

written in relative terms agrees with the observations. Therefore, self-heating will be introduced to improve the description of the effect. The significance of self-heating is evident not only in the thermal theory of initiation of explosions by Franck-Kamenetski[16] (which is concerned with temperature increase in a heated sample), but also by experimental results of Vaughan and Phillips[17] in the case of mercury fulminate.

For practical reasons, the kinetic equation (4) is rewritten in terms of concentrations normalized with respect to the initial concentration b :

$$\frac{dx}{dt} = ke^{-E/RT} . (\alpha_0 + x)(1 - x) \qquad (12)$$

where x is now the relative amount of the reacted material ; k is a proportionality factor which includes the frequency factor ; E and R represent, respectively, the activation energy and the gas constant, and α_0 is the (formal) initial concentration of the catalyst. If α_0 is interpreted as the ratio of the reaction-rate constants according to Andreev[15], α_0 will be dependent on temperature, though of a lower order than k alone. This fact may permit one to consider α_0 approximately constant compared to k.

Again, loss of heat by conduction will be neglected, which is a reasonable assumption when the self-heating is small during the main part of the induction period, as is shown later. The heat of reaction evolved by an exothermic reaction will then be used solely to raise the temperature of the explosive sample. On this basis, the amount reacted x is given by :

$$x = \frac{C_v(T - T_0)}{\Delta H} \qquad (13)$$

where T_0 is the bath or hot-plate temperature, and C_v is a mean specific heat. By introducing a hypothetical temperature T_∞ which is the maximum temperature attainable in an adiabatic reaction, equation (13) can be written :

$$x = \frac{T - T_0}{T_\infty - T_0} \qquad (14)$$

From equations (12) and (14), it follows that :

$$\frac{dT}{dt} = \frac{k . e^{-E/RT}}{T_\infty - T_0} . (T - T_1)(T_\infty - T) \qquad (15)$$

where :

$$T_1 = T_0 - \alpha_0(T_\infty - T_0)$$

Integrating equation (15), one obtains, in terms of the logarithmic integral :

$$t = \frac{1}{(1+\alpha_0)k}\left\{\left(e^{y_1}\int_{{}_1p_0}^{\infty}\frac{e^{-p}}{p}\mathrm{d}p + e^{y_\infty}\int_{-\infty}^{q_0}\frac{e^q}{q}\mathrm{d}q\right) + \right.$$

$$\left. -\left(e^{y_1}\int_{{}_1p}^{\infty}\frac{e^{-p}}{p}\mathrm{d}p + e^{y_\infty}\int_{-\infty}^{q}\frac{e^q}{q}\mathrm{d}q\right)\right\} \qquad (16)$$

where
$y = E/RT$, $y_0 = E/RT_0$, $y_1 = E/RT_1$ $y_\infty = E/RT_\infty$, ${}_1p_0 = y_1 - y_0$, $q_0 = y_0 + y_\infty$, ${}_1p = y_1 - y$, $q = y + y_\infty$.

Fig. 1 shows the temperature-time profile schematically. (The dotted line indicates the above-mentioned influence of loss of heat by conduction.) The steep rise of temperature represents a self-acceleratory reaction leading to ignition, and the time required for this to occur may be defined as the induction period τ_0, characteristic of the sample under study. Such a definition implies that near τ_0 the time is practically independent of the temperature, and consequently that the last two T-dependent terms in equation (16) compensate each other. Then the induction period:

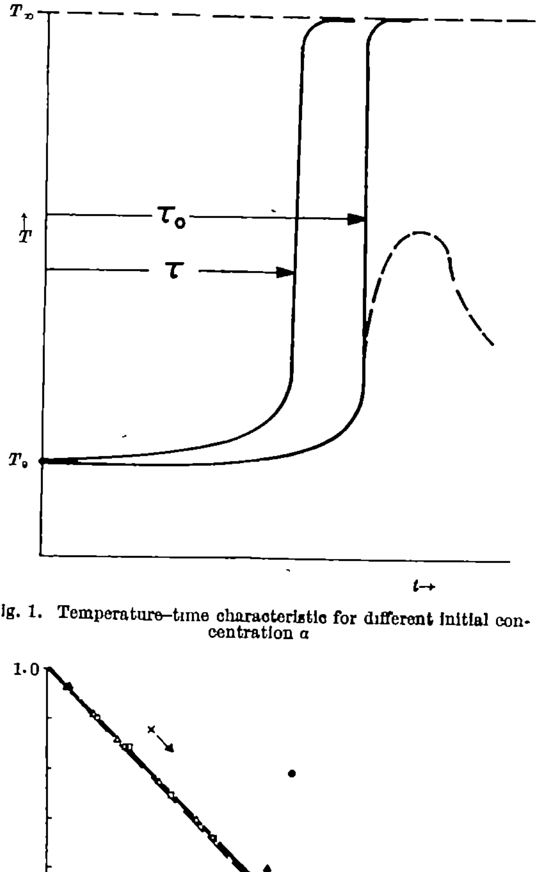

Fig. 1. Temperature–time characteristic for different initial concentration a

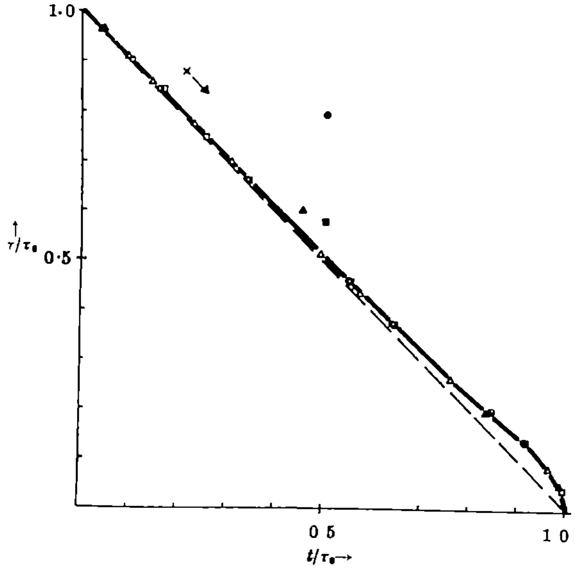

Fig. 2. Calculated pairs of values (τ/τ_0, t/τ_0) for: lead azide, △ : E = 38 kcal./mole, $\Delta H/C_v$ = 8,836° K., T_0 = 603° K.; PETN, ○ : E = 37 kcal./mole, $\Delta H/C_v$ = 5,130° K., T_0 = 521° K.; tetryl, □ : E = 52 kcal./mole, $\Delta H/C_v$ = 4,804° K., T_0 = 525° K.; in all three cases, $\alpha_0 = 10^{-6}$. The solid signs, ▲, ●, ■, represent experimental values

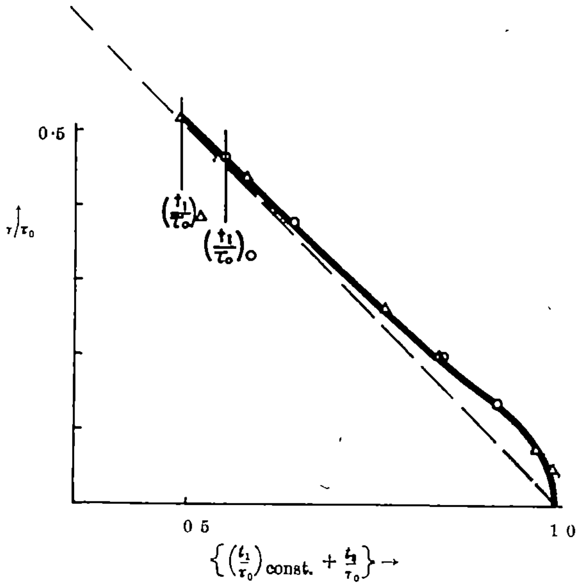

Fig. 3. Calculated pairs of values concerning the preheating times (t_1/τ_0 = const.) for lead azide (△) and PETN (○)

$$\tau_0 = \frac{1}{(1+\alpha_0)k}\left\{e^{y_1}\int_{{}_1p_0}^{\infty}\frac{e^{-p}}{p}\mathrm{d}p + e^{y_\infty}\int_{-\infty}^{q_0}\frac{e^q}{q}\mathrm{d}q\right\} \qquad (17)$$

depends, in essence, only on the initial concentration of the catalyst and the bath temperature T_0. The memory effect is now reduced to a description of the amount of autocatalytic decomposition product formed in a given length of time.

When the autocatalytic reaction is interrupted after a heating time t by cooling the explosive sample, an amount x of the catalyst formed can be calculated by using equation (16) and the relationship between x and T shown in equation (13). When the reaction is resumed by heating to the previous temperature T_0, the initial concentration of catalyst is increased by the amount x to $\alpha_1 = \alpha_0 + x$, and the new, shorter induction time τ can be evaluated by a similar integral:

$$\tau = \frac{1}{(1+\alpha_1)k}\left\{e^{y_2}\int_{{}_2p_0}^{\infty}\frac{e^{-p}}{p}\mathrm{d}p + e^{y_\infty}\int_{-\infty}^{q_0}\frac{e^q}{q}\mathrm{d}q\right\} \qquad (18)$$

where

$$y_2 = E\{R[T_0 - \alpha_1(T_\infty - T_0)]\}^{-1},\ {}_2p_0 = y_2 - y_0.$$

Knowing τ_0, τ, and t from the integrals mentioned, and assuming the constancy of k, one can check the memory effect by plotting τ/τ_0 against t/τ_0. For an exact linear memory effect, a straight line with a negative slope of unity will result. Fig. 2 shows a plot of three examples with known ΔH, E, and C_v. In all cases α_0 is chosen to be relatively small, such as

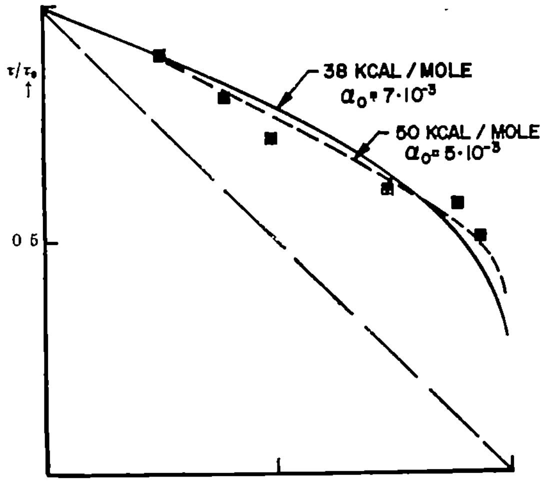

Fig. 4. Thermal sensitization of Service lead azide at 330° C. ■, Experimental values by A. S. Hawkes and C. A. Winkler, and calculated curves

10^{-6}. By this choice, the linear memory effect is still well approximated. Fig. 3 gives a demonstration of a second preheating time which causes scarcely any change of the curve (the heavy line in Fig. 3 is taken from Fig. 2).

As mentioned earlier, the experimental results for the most part do not agree with even a fairly linear relationship as also indicated by a few experimental points in Fig. 2. A theoretical curve which fits these results can be determined by changing the parameter α_0. Fig. 4 shows a comparison of memory effect data by Hawkes and Winkler[18] and two calculated curves at a constant bath temperature of 330° C. for activation energies of 38 kcal./mole and 50 kcal./mole respectively. The test material in these cases was Service lead azide, so the assumed α_0-values of 7×10^{-3} and 5×10^{-3} respectively cannot be said to be characteristic of the pure material. Nevertheless, it seems doubtful that such a relatively high α_0-value can represent lattice effects only. For lead azide, it was observed that the autocatalytic reaction is superposed by a simple, probably first-order reaction[19], in the sense used by Andreev[15]. Thus, even the degenerated, non-linear memory effect can be explained by the model demonstrated.

In summary, the mathematical treatment presented here provides a theoretical explanation of the relationship between the memory effect in an explosive and its autocatalytic decomposition as shown by the validity of equation (1) or the inequality (11), respectively.

We wish to acknowledge the encouragement of Dr. J. V. R. Kaufman.

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OCTAHEDRAL CO-ORDINATION OF NICKEL IN NICKEL(II) *BIS*ACETYLACETONE

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DISCUSSION of fourfold co-ordination of the nickel atom in paramagnetic chelate complexes of nickel(II), $Ni(x—x)_2$, where $x—x$ is a bidentate ligand such as a β-diketone or salicylaldehyde, has centred on whether such co-ordination is tetrahedral or square planar. An early suggestion[1] that magnetic data indicated the tetrahedral arrangement was rejected by Calvin and Melchior[2] as inconclusive. More recently, Maki[3] has claimed that no *a priori* correlation between the magnetic moment and structure of these complexes is possible since a molecule having, say, a square planar configuration may be either diamagnetic or paramagnetic depending critically on the strength and symmetry of the ligand field. Maki[4] indeed suggests, on the basis of the ultra-violet absorption spectrum in benzene, that nickel(II) *bis*acetylacetone, often quoted as an example of a tetrahedral complex, has a planar structure. Tetrahedral co-ordination of nickel(II) has been demonstrated for *bis*triphenylmethylarsonium nickel tetrachloride[5].

An early X-ray diffraction analysis[6] of nickel(II) *bis*acetylacetone produced the unexpected result that the molecules are trimeric; a complete solution of the structure was not possible at that time, although it was clear that the nickel atoms in the trimer might well be octahedrally co-ordinated. We now report that the complete analysis of the structure, by Fourier and least-squares methods, confirms this suggestion. The usual discrepancy factor for this kind of analysis:

$$R = \frac{\sum_{hkl} \left| |F_{\text{obs.}}| - |F_{\text{calc.}}| \right|}{\sum_{hkl} |F_{\text{obs.}}|}$$

is 0·14 at the present stage of refinement and corresponds to average estimated standard deviations of 0·015 Å. and 0·05 Å. for the nickel–nickel and nickel–oxygen bond-lengths.

A perspective diagram showing the arrangement of the ligands around the nickel atoms is shown in Fig. 1; bond-lengths and bond-angles are given in Fig. 2.

A distorted octahedral co-ordination of each nickel atom results from the bridging of each pair of nickel atoms by three oxygen atoms. The Ni(1) – Ni(2) and Ni(2) – Ni(3) separations are 2·887 Å. and 2·896 Å. respectively (equal, within experimental error). The bond-angle Ni(1) – Ni(2) – Ni(3) is 179·2° ± 0·7°; the arrangement of the nickel atoms is thus linear.

The molecule as a whole is pseudo-centrosymmetric, the root-mean-square deviation of the lengths of chemically equivalent Ni–O bonds being 0·04 Å. and of equivalent O–Ni–O and Ni–O–Ni bond-angles 3°. Since the environment of the molecule in the crystal is not centrosymmetric, there is little evidence that intermolecular forces play any substantial part in establishing the molecular configuration in the crystal. The Ni–O bond-length appears to depend on whether the oxygen is bonded to only one nickel atom ($\bar{l}_{\text{Ni—O}} = 2{\cdot}00$ Å.) or shared between two nickel atoms ($\bar{l}_{\text{Ni—O}} = 2{\cdot}12$ Å.) (see Fig. 2 for spread of observed bond-lengths).

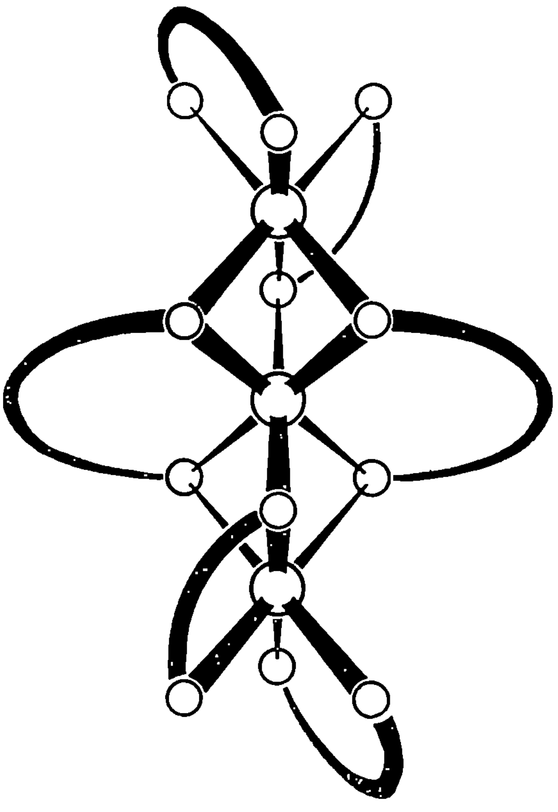

Fig. 1

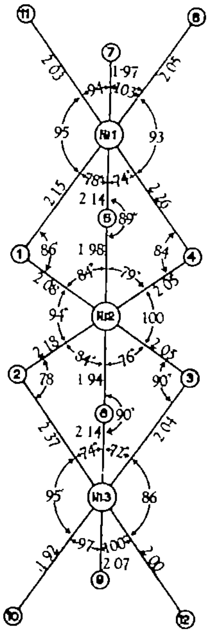

Fig. 2

The trimeric nature of the molecule is understandable in so far as a trimer is the smallest polymeric unit in which octahedral co-ordination of all the nickel atoms can be accomplished, provided there is only intramolecular sharing of oxygen atoms. It seems possible that trimerization occurs with other complexes of this type if the chelate groups are not too bulky for steric hindrance to prevent the association. A recent investigation[7] of single crystals of nickel(II) *bis*salicylaldehyde shows that the monoclinic unit cell contains twelve formula units. Zinc(II) *bis*acetylacetone crystallizes in a similar way with $a = 19{\cdot}2$ Å., $b = 8{\cdot}4$ Å., $c = 24{\cdot}4$ Å., $\beta = 113°$, and with twelve formula units in the unit cell ($\rho_{\text{obs.}} = 1{\cdot}40$ gm. cm.$^{-3}$, $\rho_{\text{calc.}} = 1{\cdot}45$ gm. cm.$^{-3}$). This implies that, as with nickel(II) *bis*acetylacetone, these structures contain trimeric units.

The present confirmation of octahedral co-ordination of the complex in the crystal makes it desirable to re-examine deductions regarding the structure of nickel(II) *bis*acetylacetone in the vapour phase and in solution. Shibata *et al.*[8,9] concluded from electron diffraction studies that the molecule is planar in the vapour phase; the analysis is inconclusive, since the possibility of octahedral co-ordination was not considered. The observed radial distribution curve does not agree well with that calculated on the basis of a planar model, and a rough calculation shows that an octahedral configuration is in reasonable agreement with the data. Isomorphism of nickel(II) *bis*acetylacetone and zinc(II) *bis*acetylacetone was also claimed[10], whereas our single-crystal X-ray studies demonstrate orthorhombic and monoclinic symmetries respectively; the identification of the present crystal structure with a yellow high-temperature modification[6] cannot be valid.

Maki favours a planar structure of the complex in solution[4]; Jørgensen[11] suggests that octahedral co-ordination results from two solvent molecules (benzene) interacting in the *trans* positions. Early molecular weight determinations in benzene made by one of us indicated molecular association; more accurate measurements (F. A. Cotton, private communication) appear consistent with a monomer–trimer equilibrium. We believe that trimerization will occur as a means of achieving octahedral co-ordination, which could also be accomplished through solvent interaction with the monomer or dimer.

The calculations for this work were carried out on the University of London *Mercury* computer. We wish to thank Mr. O. S. Mills and Dr. J. S. Rollett for making their computer programmes available to us.

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SEPARATION OF UNIVALENT FRAGMENTS OF RABBIT ANTIBODY BY REDUCTION OF A SINGLE, LABILE DISULPHIDE BOND

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PORTER[1,2] has shown that hydrolysis of the rabbit antibody molecule with papain yields three fragments, two of which possess combining sites; the third is inactive but crystallizable. He reported that the molecular weight of the inactive fragment is about 80,000, while that of each active segment is 50,000–55,000. Evidence indicating that the two active fragments are univalent is their capacity to block precipitation in the homologous system; the observation of two chromatographically separable active fragments derived from the originally bivalent molecule[1,2]; and the direct determination of valence by measurement of maximum hapten-binding capacity[3].

It has been found by Nisonoff *et al.*[4,5] that successive treatments of the antibody with pepsin at *p*H 4·8, and one of several disulphide-splitting reagents at *p*H 8, where pepsin is inactive, similarly yield univalent fragments. These split-products exhibit a sedimentation pattern and coefficient (3·5 *S*) almost identical with those of a papain digest[5], and are separated into two active and one inactive fractions on carboxymethylcellulose, under conditions identical with those used by Porter for a papain digest[1]. Proteolysis with pepsin alone causes a reduction in sedimentation coefficient from about 6·5 to 5 *S*, but the product is still bivalent, as indicated by the retention of capacity to precipitate specifically. Subsequent treatment with a reducing agent or cyanide yields fragments with $s \simeq 3{\cdot}5$. Strong evidence indicating that these fragments are univalent is discussed in ref. 5. It was therefore concluded[5] that the transformation from bivalent to univalent antibody was accomplished by the splitting of one or more disulphide bonds.

Since papain, being a sulphydryl enzyme, has always been used in conjunction with a reagent capable of splitting disulphide bonds, it appeared that a similar mechanism may exist in the digestion of rabbit antibody by that enzyme; that is, removal of an inactive fragment by proteolysis, followed by splitting of the bivalent residue through reduction of one or more S—S bonds.

Recently it has been found[6] that the molecular weight of the 5 *S* precipitating fragment formed by the action of pepsin alone is 106,000, while the average molecular weight of the univalent split-products formed by the further action of a disulphide-splitting reagent (2-mercaptoethylamine) is 56,000.

In the present report data are presented indicating that this transformation can be accomplished by reduction of a single, critical disulphide bond. The results further indicate that this bond is exceptionally labile.

Three gm. of a γ-globulin fraction[7] of rabbit anti-ovalbumin was treated with 2 per cent of its own weight of pepsin at *p*H 4·5 in sodium acetate–chloride buffer of ionic strength 0·14. After incubation for 18 hr. at 37°, the mixture was adjusted to *p*H 8 and dialysed against 4 litres of cold saline–borate buffer, *p*H 8, ionic strength 0·16. The major component was isolated by precipitation with sodium sulphate[6]. On examination in the ultracentrifuge a single peak was observed with $s_{20w} = 5{\cdot}1$. The yield of protein was 1·6 gm. or 53 per cent of the weight of γ-globulin initially present. In the procedure the final dialysis was carried out against 0·06 *M* sodium acetate adjusted to *p*H 7 with acetic acid.

Two-ml. aliquots of this preparation, containing 34 mgm. protein per ml., were adjusted to *p*H 5·0 with 2 *M* acetic acid and treated with varying amounts of 2-mercaptoethylamine hydrochloride (MEA) at this *p*H. Each reaction was allowed to proceed for 1 hr. at 37°. At the end of this period 1 ml. of 10 per cent trichloracetic acid was added to each of two 0·5-ml. aliquots taken from a reaction mixture. After standing for approximately ½ hr. each mixture was centrifuged and the precipitate was washed with two 1–2 ml. portions of 5 per cent trichloracetic acid. (It was found that MEA is soluble in 5–10 per cent trichloracetic acid.) The precipitate was then suspended uniformly in 1·2 ml. of saline–borate buffer, *p*H 8, ionic strength 0·16. A 0·1-ml. aliquot was removed for analysis of the protein concentration, and 1 ml. of suspension was dissolved in 30 ml. of the buffer used in the titration of sulphydryl groups[8]. The protein dissolved readily in each case and titrations were carried out immediately. 81–87 per cent of the protein was precipitated by trichloracetic acid in each experiment. More protein was precipitated from the supernatants during the next hour or two, but titrations were carried out early to minimize the possibility of re-oxidation of SH groups. Calculations of the number of SH groups per molecule were based on amounts of protein actually precipitated. As indicated, the procedure yielded duplicate values for the SH content of each sample.

Titration of sulphydryl groups was carried out amperometrically according to the method of Benesch, Lardy and Benesch[8] using 4×10^{-3} *M* silver nitrate. To increase the availability of SH groups a detergent, 0·5 per cent 'Duponol *C*', was added to the titration mixture[9]. In several preliminary experiments it was found that doubling the detergent concentration caused no further increase in the number of titratable SH groups.

A control was run by precipitating the protein with trichloracetic acid without prior treatment with 2-mercaptoethylamine. This was done in triplicate and gave a mean value of 0·75 mole of SH groups per mole of protein (molecular weight

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† Established investigator of the American Heart Association.

106,000), with a mean deviation of 0·09 mole of SH.

For the purpose of testing the effect of reduction on the sedimentation characteristics, aliquots of each mixture, after the reaction with MEA, were quickly cooled to room temperature and treated immediately with enough 1 *M* iodoacetamide to yield a final concentration of 0·04 *M*. In each case this represented an excess in molar concentration over that of the MEA present. The solution was adjusted to *p*H 7·0 and diluted to a protein concentration of 10 mgm./ml. with 0·1 *M* sodium acetate containing sufficient acetic acid to lower its *p*H to 7·0. Samples were then examined in a Spinco model *E* ultracentrifuge at a speed of 59,780 r.p.m. Runs were started within an hour after completion of the reaction with 2-mercaptoethylamine, except in the case noted below, where the effect of standing after treatment with iodoacetamide was tested.

The effect on the sedimentation pattern of a 1-hr. treatment with increasing amounts of MEA is shown in Fig. 1. The s_{20w} values are indicated on the diagrams. With 0·014 *M* or 0·03 *M* MEA the breakdown to $s_{20w} \simeq 3·5$ appeared to be complete. With 0·006 *M* MEA 83 per cent of the protein was broken down, while the transformation reached 66 per cent of completion with 0·004 *M* MEA. The latter run was repeated in the ultracentrifuge after the mixture had been allowed to stand overnight at room temperature. There was no significant change in the pattern (fourth photograph, Fig. 1).

The correlation of these results with the number of new sulphydryl groups formed per molecule is indicated by the graph in Fig. 2. Complete breakdown of the 5 *S* protein was associated with the formation of between 1·7 and 2·9 moles of SH groups per mole of protein of molecular weight 106,000, which corresponds to the splitting of more than 0·85 and less than 1·45 disulphide bonds per molecule.

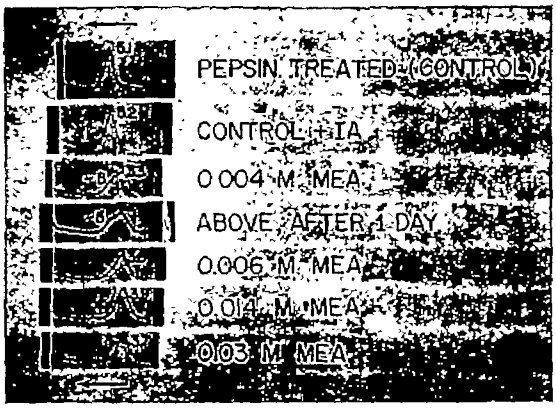

Fig. 1. Effect of 2-mercaptoethylamine hydrochloride (MEA) on the sedimentation characteristics of a purified peptic digest of rabbit antiovalbumin γ-globulin. Photographs taken 67 min after attainment of full speed (59,780 r.p.m.) in the Spinco model *E* ultracentrifuge. The abbreviation IA refers to iodoacetamide. Each sample except the control contained 0·04 *M* iodoacetamide. Each solution also contained 0·1 *M* sodium acetate, adjusted to *p*H 7 with acetic acid. Details of procedures are given in the text The S_{20w} value is indicated near each peak

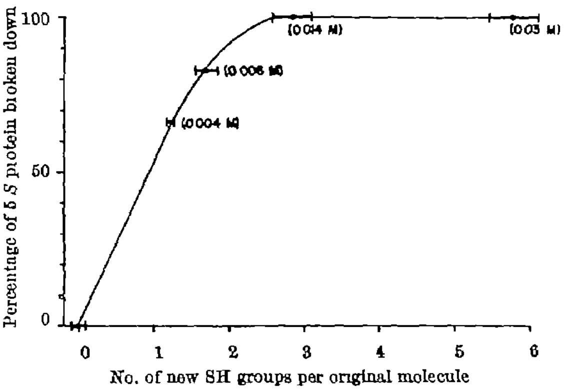

Fig. 2. Effect of 2-mercaptoethylamine hydrochloride (MEA) on a purified peptic digest of rabbit antiovalbumin γ-globulin. The molarity of MEA used is given on the graph. Each reaction was carried out for 1 hr. at 37° as described in the text

It is of interest to compare the fraction of 5 *S* protein broken down with the fraction of a disulphide bond per molecule split, for the experiments in which the breakdown was incomplete. The assumption is made that two new SH groups correspond to the reduction of one disulphide bond. The respective values are 0·66 versus 0·61 (0·004 *M* MEA), and 0·83 versus 0·85 (0·006 *M* MEA). The results indicate that the conversion from a sedimentation coefficient of about 5 *S* to 3·5 *S* is associated with the reduction of a single disulphide bond, and that this bond is exceptionally labile. (If the assumption is made that the 5 *S* protein consists of Porter's fractions I and II, his amino-acid analyses[2] would indicate that there are about 10–12 S—S bonds in the molecule.) Previous measurements of molecular weights further indicate that the molecule is split roughly in half during this conversion[6].

Another characteristic of the conversion is that the molecule loses its capacity to precipitate specifically and acquires the ability to block precipitation in the homologous precipitin reaction[4,5]. There is strong evidence that this is attributable to the univalence of the fragments resulting from treatment with a disulphide-splitting reagent[5]. In the present experiments a portion of each sample tested in the ultracentrifuge was dialysed overnight against 4 litres of the cold saline–borate buffer described above. The protein concentration was adjusted to 8 mgm./ml. and 0·25 ml. portions (2 mgm.) were tested in duplicate for their effect on the precipitation of 1·4 mgm. of untreated antiovalbumin γ-globulin with slightly less than an optimum concentration of ovalbumin, in saline–borate buffer, *p*H 8. Each solution contained 40 μgm. of antigen and the total volume was 0·65 ml. Mixtures were refrigerated for five days. In the absence of fragments, 335 ± 7 μgm. of precipitate was formed. In the presence of the samples treated with 0·006 *M*, 0·014 *M* or 0·03 *M* MEA, no precipitate was formed. The sample treated with 0·004 *M* MEA caused a reduction from 335 to 265 ± 6 μgm. of precipitate. This partial blocking may be ascribed to the fact that the sample was only partially reduced.

None of these preparations caused any significant inhibition in a heterologous reaction, that of bovine γ-globulin reacting with its rabbit antibody to yield 310 μgm. of precipitate. One of the preparations (reduced with 0·006 *M* MEA) caused an increase of 25 per cent in the amount of precipitate formed in the heterologous system. The reason for this apparent enhancement is not known.

None of the MEA-treated samples formed a precipitate when mixed with an amount of antigen that was optimal for the untreated 5 *S* protein, or with one-fourth this quantity of antigen. The limited amount of material available prevented further testing. However, previous work has shown that the 3·5 *S* protein does not form specific precipitates when tested with a wide range of antigen concentrations[8].

In another control experiment it was found that treatment with 0·04 *M* iodoacetamide at *p*H 5, followed by dialysis, had no significant effect on the amount of precipitate formed by a given weight of the pepsin-treated antibody preparation used in these experiments.

There is considerable evidence[5] suggesting that the univalent fragments formed by reduction of the peptic digest of rabbit antibody correspond to Porter's fractions I and II. The present results suggest that these fractions are linked in the antibody molecule by a single, highly reactive disulphide bond. The results do not preclude the possibility that additional bonds, hydrolysed by pepsin, also link these sub-units, or that other, weaker bonds may be involved.

This work was supported by Grant *E*-2858 from the National Institutes of Health.

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EFFECTS OF ŒSTROGEN ON SOMATIC VARIATION OF AN ERYTHROCYTE AGGLUTINOGEN IN CHICKENS

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SOMATIC variation of a permanent nature may arise from admixture of erythropoietic tissue during fœtal life[1]. More recently, somatic mutation has been proposed to explain the presence of a low frequency of inagglutinable cells in a population composed predominantly of agglutinable cells[2,3]. Somatic variation may also arise from events of a more transient nature, such as the coating of red cells by plasma as occurs in the '*J*' substance of cattle[4] and the '*R*' substance of sheep[5]. Evidence has been obtained that a hormone also plays a part in somatic variation of a red cell agglutinogen.

A survey of 69 seed extracts which could be used as typing fluids for chicken red cells was carried out with the following results: 64 per cent did not react with the red cells of any of the chickens tested; 9 per cent agglutinated the red cells of all chickens tested; 5 per cent hæmolysed the red cells of all chickens tested and 22 per cent detected differences among individual chickens, 20 or more birds being used in these tests. Among the extracts which could distinguish the cells of individual chickens, those from *Lathyrus cicera*, the falcon pea, and *Pisum arvense*, the Canadian field pea, gave identical reactivities with all birds tested; the substance detected by these extracts was designated 'H_1'. These extracts were found to agglutinate only the red blood cells of females. There were 54 males tested and all were found to be without reactivity; however, the cells of 75 of the 129 females tested were found to be reactive. Reactivity on the red cells was detected when the females reached sexual maturity. Among 7 females tested all became reactive after the onset of egg-laying and maximum macroscopic agglutination scores were reached from 14 to 31 days.

A number of correlative studies (62) were carried out in which chickens were typed monthly or more frequently and the cumulative egg production recorded from one test period until the next. On the basis of these results the hens were placed in three classes; those which were laying or which had laid and whose cells were reactive; those that had never laid and whose cells had never shown reactivity; and the third class consisting of those hens whose cells were non-reactive regardless of the condition of laying. One bird was found the cells of which were reactive but which had not laid any eggs. An autopsy of this bird showed that she was an internal layer, for there were a large number of yolks in the body cavity; therefore, this bird properly belongs in the first category.

Table 1. COMPARISON OF REACTIVITIES OF TERMINAL RED CELLS WITH BONE MARROW CELLS OF NO. *M*5792

	L. cicera		*P. arvense*		Saline or egg albumen	
	Terminal red cells	Bone marrow cells	Terminal red cells	Bone marrow cells	Terminal red cells	Bone marrow cells
11 days after ovariectomy	2–3	(+)	3–4	+	0	0
28 days following ovariectomy	0	—	0	—	0	0
4 days after treatment with diethylstilbœstrol	(+ – 1)	2–3	(+ – 1)	3–4	0	0
	In titrations with *P. arvense*					
	Undiluted	1 2	1 4	1 . 8	1 · 16	1 : 32
11 days after ovariectomy						
Bone marrow cells	+	+	+	(+)	0	0
Terminal red cells	–4	3–4	2–3	2–3	2	0
4 days after treatment with diethylstilbœstrol						
Bone marrow cells	2–3	2–3	2–3	1–2	(1–2)	0
Terminal red cells	(+)	(+)	*	0	0	0

Dashes indicate that tests were not carried out; values in parenthesis are microscopic scores; * refers to a questionable reaction.

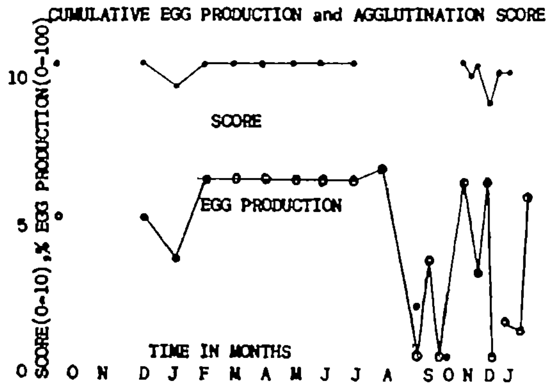

Fig. 1

Fig. 1 presents the results obtained over a period of months with one of the 62 birds under investigation; where the line is not continuous this indicates that information either on agglutination or egg production was not obtained for that date. It is evident that there is a relationship between egg-laying and the presence of the agglutinogen, since the score rises and falls when the egg production rises and falls and remains constant when the egg production is constant. There is a lag, however, between change in score and change in egg production which is most clearly seen for the month of January, when weekly and bi-weekly testing was carried out. A similar lag was observed when birds began egg-laying. The present hypothesis is that the lag was a consequence of transformation to agglutinability which occurs in the bone marrow. Evidence for this hypothesis is presented in Table 1. As expected, under the replacement hypothesis bone marrow was found to be less reactive when the bird was ovariectomized and the terminal red cells were decreasing in reactivity but were still reactive, and, conversely, when the terminal red cells were low in reactivity immediately following the injection of œstrogen, the bone marrow cells were more reactive. Similar experiments have been carried out with other birds, and analogous results were observed in birds beginning to lay or going out of egg production.

The basis for the association between egg-laying and the occurrence of the 'H_i' substance appears to be hormonal. In Fig. 2 it may be seen that the agglutination score decreases to zero following ovariectomy. When injections of diethylstilbœstrol were made, agglutinability increased and when injections of the hormone were discontinued the agglutinability of cells declined. Injection of corn oil (the hormone solvent used) in an ovariectomized bird did not result in a rise in agglutinability, whereas injections of hormones in two other ovariectomized birds gave marked increases in agglutinability as observed with No. *N*5792. All of these results paralleled the increase in agglutinability observed when birds began to lay or were coming back into egg production following a pause.

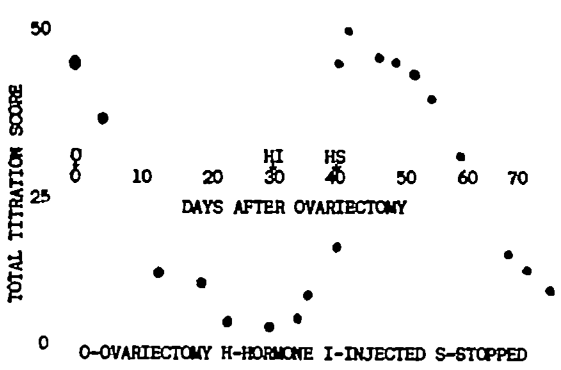

Fig. 2

Preliminary results from pedigree data indicate that the variation observed among the females with respect to the agglutinogen 'H_i' also has a familial basis. Pedigree data were obtained from laying birds the red cells of which were non-reactive and from laying birds the cells of which were reactive. Matings of non-reactive hens from a highly inbred line, from which all the hens tested were non-reactive, gave rise to 29 female progeny, all of which were non-reactive. Four other hens from the same line which were reactive gave rise to 29 female progeny, all of which were non-reactive. Four other hens from the same line which were dead at the time of typing had a total of nine female progeny all of which were non-reactive. The three sires were from lines in which the females were non-reactive and one male was closely related to all the dams. Since it had been shown that diethylstilbœstrol can cause an increase in agglutinability in females which were known reactors, it was of interest to determine whether the presumed genetically non-reactive birds could become reactive if the œstrogen-level were increased. Two females from the non-reactive progeny which were laying were given 16 injections of diethylstilbœstrol, each injection consisting of 0·53 mgm.; a third female, also laying from the same group, was given corn oil. The red cells of these three birds have remained non-reactive during the entire period of testing. These results suggest that there may also be differences in the red cells of the reactive and non-reactive birds not primarily due to the influence of hormone.

Some results were also obtained from test-cross types of matings which demonstrate segregation in the offspring of some reactive hens. Sires were from families in which the female sibs or maternal parent were reactive and such males were mated to non-reactive hens. The reciprocal matings were also made. A total of seven sires were mated to eleven dams and from these matings 12 reactive and 18 non-reactive female progeny were obtained. Results were also obtained from matings (4 sires, 14 dams) in which the hens were not available for typing (they were either dead or had been culled) and the males were from lines in which the hens were reactive. Such matings produced 14 reactive female progeny, 18 non-reactive female progeny and 3 progeny of questionable reactivity.

These observations suggest that the variation observed among the laying hens with respect to the 'H_i' substance is a function of the genotype and a hormonal factor extrinsic to the red cells.

The results presented here will be treated more fully elsewhere. We thank Prof. M. R. Irwin for suggestions in the preparation of this communication.

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LETTERS TO THE EDITORS

PHYSICS

Space-Charge Currents in Gallium Arsenide

Mott and Gurney[1] pointed out that an insulator should be able to carry an electronic current if electrons were injected into it by a suitable contact. Such currents were observed experimentally by Smith and Rose[2] in cadmium sulphide crystals. They showed that an essential condition was that the 'deep trap' density should be low, deep traps being centres with energy-levels well below the conduction band which can accept electrons. In a recent communication[3] a model was proposed to account for the properties of semi-insulating gallium arsenide. The model suggested that in this material the above condition should be satisfied, and as a result it was predicted that space-charge currents should be observable. Here we present experimental results confirming the correctness of the prediction.

Wafers of semi-insulating gallium arsenide were prepared from bulk ingots by cutting, grinding and etching. Typical dimensions were 3 × 3 × 0·15 mm. Tin contacts of 0·5 mm. diameter were alloyed at the centres of the two large faces. This configuration makes any surface leakage paths between contacts long. A current-voltage plot taken under d.c. conditions at room temperature for one such specimen is shown in Fig. 1. In the region in which current increases rapidly there were some changes with time, and Fig. 1 shows steady-state values.

Lampert[4] has given the theory for space-charge currents when a single trap-level is present, and we may readily adapt his theory to our model for gallium arsenide. At low voltages any injected charge is trapped, and to a good approximation Ohm's law is obeyed. At high voltages space-charge due to traps is small compared with that due to injected carriers and the variation of current with voltage is given by the solid-state analogue of Child's law[1]:

$$I = \frac{9}{32\pi}\frac{\varkappa\mu V^2}{L^3} \qquad (1)$$

The ohmic and space-charge regions are connected by a region in which the current increases by several orders of magnitude for a small change of voltage. The voltage at which this occurs, called by Lampert the 'traps-filled-limit' voltage, is just the change in potential produced by the space-charge of the filled traps, that is:

$$V_{TFL} = \frac{2\pi e}{\varkappa}L^2(N_A - N_D) \qquad (2)$$

For the specimen the characteristic of which is shown in Fig. 1 the magnitude of the space-charge current agrees with that given by equation 1 within a factor three, which is within the limits of experimental error. Since in this case the thickness L was 0·12 mm., the unfilled trap density $N_A - N_D$ is found from equation 2 to be 8×10^{10} cm.$^{-3}$.

For another specimen from a different ingot the value V_{TFL} was measured and found to be 60 V. Mr. N. A. C. Thompson, of this Laboratory, has measured the resistivity and Hall effect as a function of temperature for specimens cut from the same ingot. From equation 2 we determine $N_A - N_D$ at room temperature to be $3 \cdot 8 \times 10^{12}$ cm.$^{-3}$. Applying the analysis of ref. 3 to Mr. Thompson's measurements, we find $\chi - 1$, which measures the proportional departure from exact compensation, to be 5×10^{-6}. Now:

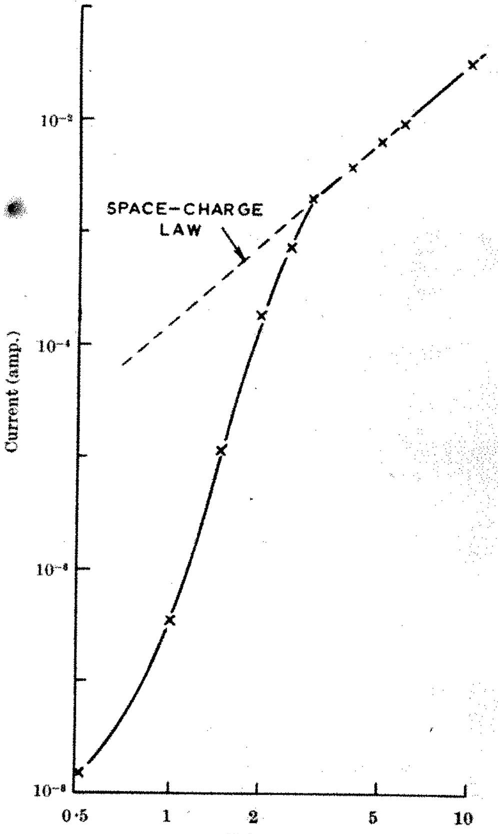

Fig. 1. Current-voltage curve for gallium arsenide showing ohmic and space-charge regions

$$N_D = \frac{N_A - N_D}{\chi - 1} \qquad (3)$$

so by combining space-charge and Hall effect measurements we find N_D, which for this particular specimen turns out to be 8×10^{17} cm.$^{-3}$, a rather high value but not improbably high.

Thus a model in which acceptors at a level deep in the energy-gap are auto-compensated by shallow donors seems a reasonable first approximation in explaining not only the Hall effect but also the space-charge currents in semi-insulating gallium arsenide. It would be interesting to know if a similar mechanism could explain the low trap density observed in cadmium sulphide, but the relevant measurements of carrier concentration as a function of temperature do not seem to be available.

We wish to express our thanks to Mr. N. A. C. Thompson for the use of his experimental results. Acknowledgment is made to the Admiralty for permission to publish this communication.

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Hyperfine Splitting of Rubidium-87

RUBIDIUM has been used in the development of gas cell frequency standards, and from measurements of the variation of frequency with the pressure of the buffer gas used in the cell it has been deduced[1] that the frequency corresponding to zero gas pressure is :

6 834 682 608 c./s. ± 7 c./s.

where the second of time is that giving 9 192 631 770 c./s. for the cæsium hyperfine structure splitting at zero field[2]. It has been found, however, that when cæsium is used in a gas cell the extrapolated frequency is 30 c./s. lower than the value given above obtained by an atomic beam experiment[3]. There is therefore considerable interest in the atomic beam value for rubidium. For this purpose the oven of the long beam chamber[4] at the National Physical Laboratory was filled with a mixture of rubidium-87 and cæsium-133. A strong beam of atoms was obtained at an oven temperature of 120° C., and the application of the appropriate radio frequency produced, alternatively, resonances of the rubidium or cæsium atoms. The amplitude of the cæsium resonance was 50 per cent greater than that of rubidium, the higher vapour pressure of cæsium presumably compensating for the fact that a greater proportion of the rubidium atoms take part in the resonance. In both cases the signal to noise ratio was adequate for the precise measurement of the frequencies corresponding to the resonance peaks. The rubidium resonance, which was 71 c./s. wide, was excited in cavities independently tuned and separated by a distance of 254 cm. It was slightly asymmetrical, but the asymmetry was measured and a correction applied. The value on the atomic time-scale corrected for the asymmetry and magnetic field is :

6 834 682 614·0 ± 1 c./s.

It is proposed to continue measurements of the two atomic transition frequencies.

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Whisker Growth from Quartz

WHISKER growth and associated phenomena have been extensively reviewed recently by Messrs. N. F. R. Nabarro and P. J. Jackson[1]. They cited observations of growth from metallic oxides, sulphides and hydroxides along with the more usual growth in metals. Whisker growth from fused quartz, apparently an unreported phenomenon, is unusual due to the vitreous nature of the substrate.

The whiskers are shown in Fig. 1. Large single whiskers and clusters of shorter ones grew from discrete places on the substrate. The quartz was heated in a 'Zirco' tube through which flowed 1 litre/min. of dry nitrogen. The temperature was approximately 1,425° C. and time for the growth was 24 hr. Immediately adjacent to the whiskers was a dense patch of pine tree-like dendrites. The whiskers ranged in length from millimetre to micron size.

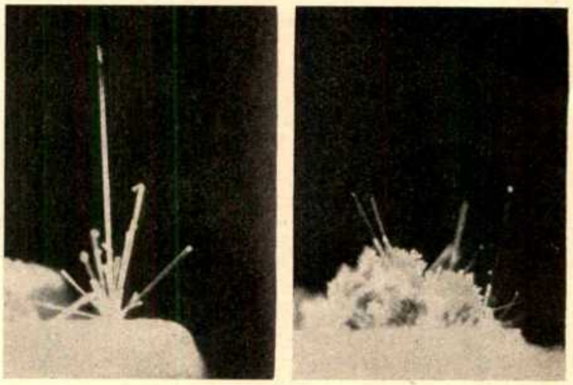

Fig. 1. Quartz whiskers. (× 15)

The quartz substrate from which these whiskers grew had a skin of devitrified quartz covering its surface due to regular use at elevated temperatures. There was silicon material placed on the substrate near the growth area. There is no apparent evidence to point to how the whiskers grew either from the base or at the tip. However, the devitrified skin afforded the conditions for nucleation.

Further work and mechanical tests will be undertaken on these whiskers.

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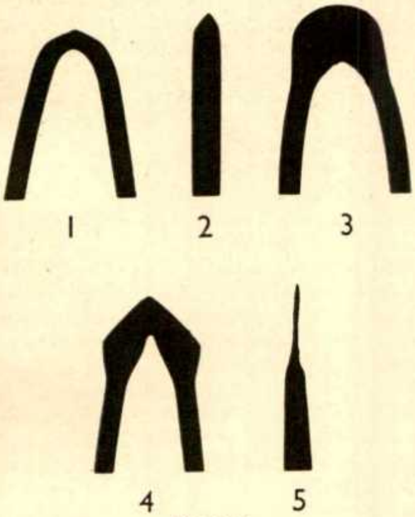
Simple Methods for preparing Pointed Filaments for the Electron Microscope

THE performance of electron guns used in the electron microscope has been studied by Haine and Einstein[1], Boersch and Born[2], and others. The present indications are that pointed filaments improve this performance. The first of these filaments was prepared by Hibi[3] by hand-polishing or etching a short length of tungsten wire which was welded or otherwise attached to a conventional hairpin filament according to Müller's method[4]. Heating was obtained by conduction from the hairpin.

The main disadvantage of the Hibi filament is that it is difficult to prepare and much may depend on the skill of the operator. The present communication describes a very simple method by which pointed filaments may be quickly prepared by any operator.

The basis of the method is to make a point on a conventional hairpin filament. This may be achieved in two ways, either by straightforward grinding or by pinching to make the filament thinner at the tip, followed by grinding. Details of the methods are as follows:

(1) A small disk of fine emery paper (grade 'Corosil' 600 or 000), about 3 cm. in diameter, is stuck to a metal disk and fitted to an electric motor which can revolve at 1,000–3,000 r.p.m. Four flats are ground, according to Figs. 1 and 2. The angles are not critical but should be about 120° in the front view (Fig. 1) and 70° in the side view (Fig. 2). Grinding is facilitated if a low-power lens is used and the following sequence is adopted: (*a*) Grind the faces in Fig. 1 so that a sharp point is obtained. Material is removed rapidly in this case. (*b*) Grind the faces in Fig. 2. More time is required to produce a point in this case. (*c*) Inspect under a × 10 lens, and if the point appears blunt from the front (Fig. 1) touch the faces on the revolving disk for a very short time. (*d*) Inspect the filament from the side (Fig. 2) and if this point appears blunt regrind very lightly.



Figs. 1–5

With a little practice a sharp point can be obtained in about 2 min. Care must be taken not to grind away more than half the diameter of the wire or premature burn-out will occur. This type of filament will be referred to as a ground filament.

(2) A much sharper point can be obtained by pinching a filament (not pre-heated *in vacuo*) at the tip before grinding, using a pair of smooth-faced pliers; the filament tip is inserted so that only about 0·5 mm. is gripped by the pliers. After exerting a sufficiently high pressure the tip will be plastically deformed to the shape shown in Figs. 3 and 5, the latter showing that it is in the form of a thin foil. Before removing the filament from the pliers it is desirable to ensure that the deformed tip has not been bent over, otherwise it must be straightened. It is important that the pliers should be properly hardened, otherwise the tip will not be flattened sufficiently.

Grinding is carried out with the same assembly as before, but it is desirable to use a finer grade of emery paper. A suitable sequence is as follows: (*a*) Touch the foil faces against the rotating wheel to produce the fine point shown in Fig. 5. (*b*) Grind a point subtending an angle of about 90° on the foil periphery (Fig. 4). (*c*) If the point shown in Fig. 5 has become blunt on inspection with a × 10 lens, regrind very lightly. (*d*) Retouch the other two faces if necessary.

The process can be carried out in 2 or 3 min., as the grinding is easier to carry out than in the first method. These filaments will be referred to as pinched filaments.

Consideration of the following general points may help to ensure satisfactory results: The lens used to view the grinding wheel must provide a magnification of about × 5. A telescope is satisfactory, provided that the image is not inverted, otherwise the handling of the filament becomes very difficult. The use of a rheostat to vary the motor speed is valuable if there is a tendency to grind away too much material from the tip. If a large number of filaments are to be made, it may be convenient to make up a simple jig with a swinging arm to hold the filament. Mounting the filament in a metal block will facilitate handling and improve grinding accuracy if a jig is not to be used. Filaments with a sharp bend at the tip give best results.

It is obvious that there is little flexibility in the way a filament can be ground, except perhaps in the case of the pinched type. Here some variation of angle

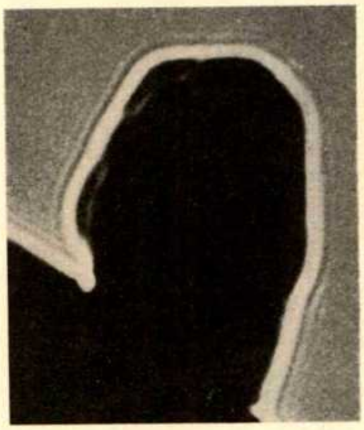


Fig. 6

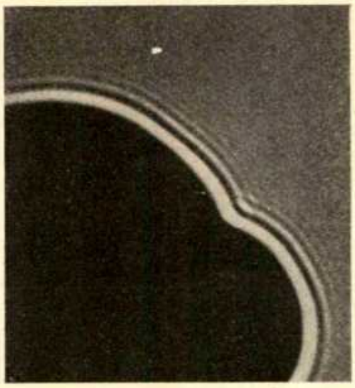


Fig. 7

and amount of material removed can be achieved, though generally with little effect on performance.

Before use, both types of filament should be heated to 2,500° C. *in vacuo*. It is, of course, very important that nothing should be allowed to touch the point, otherwise it will be damaged and the efficiency of the filament impaired.

The centring of the Hibi type of filament is critical, and this is also the case with ground and pinched filaments. All filaments have a tendency to move to a certain extent so that, particularly during the first few minutes of operation, it will be found that the electron gun requires frequent re-alignment. Some trouble can be saved by turning both filament and H.T. supplies on for a few minutes before operation ; this will give the filament a chance to settle into a stable position.

The Hibi filament provides increased coherence in the electron beam, which is also the case with ground and pinched filaments. This is demonstrated by Figs. 6 and 7, which are electron micrographs (×180,000) of under-focused Fresnel fringes taken with standard and pinched filaments respectively. Both condenser lenses were switched off for these experiments in order to have easily reproducible illumination conditions. On the original prints, three fringes were visible with the standard filament, and five with the ground and pinched types.

The increased brightness may be due to either an increased source temperature or to a reduction in space-charge effects on the emission. The temperatures of these pointed filaments have not yet been measured so that a full explanation cannot be attempted. In general, qualitative observations agree with the detailed results of Swift and Nixon[5].

I am grateful to Mr. T. Mulvey for valuable help and discussion, and also to Dr. T. E. Allibone, director of the Laboratory, for permission to publish this communication.

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METALLURGY

Production and Mobility of Point Defects in Titanium and Zirconium

ELECTRICAL resistivity measurements have been made by many workers on quenched, irradiated or plastically deformed metals, in order to study the production of defects by these various treatments. By observing the recovery of the resistivity on annealing, it is possible to study the mobility of the various defects out of the material or to internal sinks. In this communication we present some results on the mobility of defects, introduced by low-temperature plastic deformation in titanium and zirconium.

Polycrystalline titanium and zirconium wires (of a purity of about 99·9 weight per cent), were strained in tension at 78° K., and the extra resistivity introduced by the plastic strain was measured at the test temperature as a function of the strain. The increase in resistivity expressed as a percentage of the original resistivity at 78° K. was approximately 8 per cent for titanium and 5 per cent for zirconium, both due to a tensile strain of 6 per cent. Strained wires were then annealed for 15 min. at 78° K., 195° K. and room temperature, the resistivities after each anneal being measured at 78° K. After the room-temperature anneal, more than 50 per cent of the extra resistivity introduced by the plastic deformation was annealed out in the case of titanium, whereas the corresponding value for zirconium was 30 per cent. These recoveries of resistivity showed that there was appreciable mobility of defects below room temperature, these having been produced by plastic deformation. In the case of zirconium, such mobility has been observed previously for defects produced by irradiation ; for Zartman[1] has bombarded zirconium with deuterons at 170° K. and observed a 60 per cent recovery of electrical resistivity on annealing at room temperature.

A very surprising feature of this work was that, for titanium, the resistivity was in fact recovering at the temperature of deformation, 78° K. This effect was not observed in zirconium, but we believed that in this case the recovery might occur too rapidly to be detected by annealing tests at the temperature of deformation.

To test this possibility, wires of both titanium and zirconium were strained in tension at 20° K., and the extra resistivity introduced by the plastic strain was measured at 20° K. as a function of the strain. The increase in resistivity expressed as a percentage of the original resistivity at 20° K. was approximately 30 per cent for titanium and 60 per cent for zirconium, both due to a tensile strain of 6 per cent. The wires were then annealed for 15 min. at 20° K., 78° K. and room temperature, the resistivities after each anneal being measured at 20° K. It was observed, both for titanium and zirconium, that whereas there was apparently no recovery at 20° K., approximately 20 per cent of the resistivity introduced by the 20° K. deformation was recovered after the 78° K. anneal.

This recovery, at temperatures below 78° K., of resistivity introduced by plastic deformation in the hexagonal metals titanium and zirconium, is in marked contrast to the behaviour of the face-centred-cubic metals, copper, nickel and gold, where there is essentially no recovery of resistivity after plastic deformation at low temperatures[2]. In these metals, recovery of resistivity below 78° K. is only observed after very heavy deformation, when some slight recovery does occur in the temperature interval up to 78° K., the recoverable fraction of the resistivity increasing with increasing strain. Of course, there is substantial annealing in the face-centred-cubic metals below 78° K. (the so-called stage I), when the damage giving rise to the resistivity is caused by irradiation with electrons, deuterons or neutrons[3].

Although it may not be altogether valid to compare energies for the movement of various defects in the hexagonal and face-centred-cubic structures, or to compare the temperature ranges in which they move, it is interesting to speculate why there is a difference between the two structures. It would seem that, in our experiments, isolated interstitial metal atoms are produced by plastic deformation, and that these then migrate at the low temperatures involved; whereas in the case of the face-centred-cubic metals the experimental evidence indicates that they are not produced[4]. This difference might be related to a difference in stacking fault energies, for in the hexagonal case where slip occurs mainly on the $\{10\bar{1}0\}$ prism planes, the dislocations should have a relatively high stacking-fault energy, probably much higher than copper, nickel or gold. Support for this idea is provided by the results obtained for aluminium[5], a face-centred-cubic metal with a very high stacking-fault energy; for Sosin and Koehler have found that if aluminium single crystals are deformed in tension to an elongation of 15 per cent at 4° K., then there is a 10 per cent recovery of resistivity on annealing at 78° K. One would expect the effect to be much greater for poly-crystals, where the operation of intersecting slip systems can cause increased production of point defects.

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Initiation of Fatigue Cracks in Beta-Brass

FATIGUE cracks have been seen to be initiated in slip bands, twin boundaries and grain boundaries, in a number of investigations on the fatigue behaviour of metals[1-3].

During recent work on the fatigue behaviour of beta-brass a different mode of fatigue crack initiation has been observed, which has not been reported previously in the literature. Certainly, it does not occur in the case of fatigued copper.

Beta-brass was chosen because of the lack of knowledge of the fatigued behaviour of body-centred cubic metals. Polycrystalline material, with an average grain-size of 2 mm., has been fatigued in reverse plane bending at a frequency of 8,400 c./min. Specimens in the form of cantilevers were mechanically polished with care and finally electro-polished prior to fatiguing.

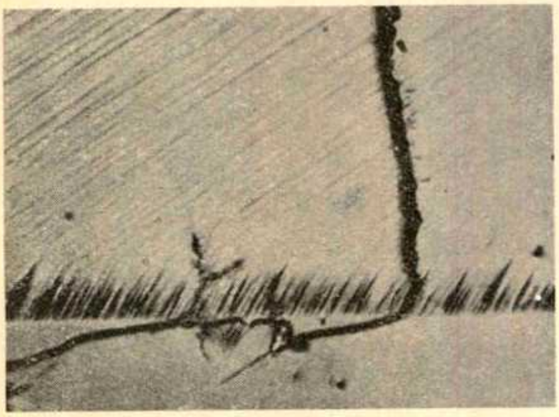

Fig. 1. Surface of fatigued beta-brass specimen. Fatigue cracks initiated in short slip markings adjacent to one side of a grain boundary. Oblique light. (× c. 1,860)

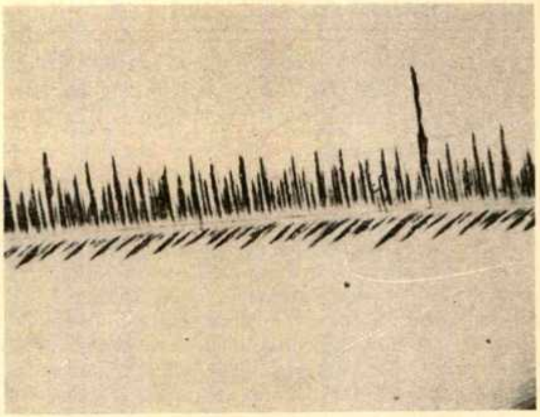

Fig. 2. Surface of fatigued beta-brass specimen. Extrusions on short slip markings adjacent to both sides of a grain boundary. (× c. 1,070)

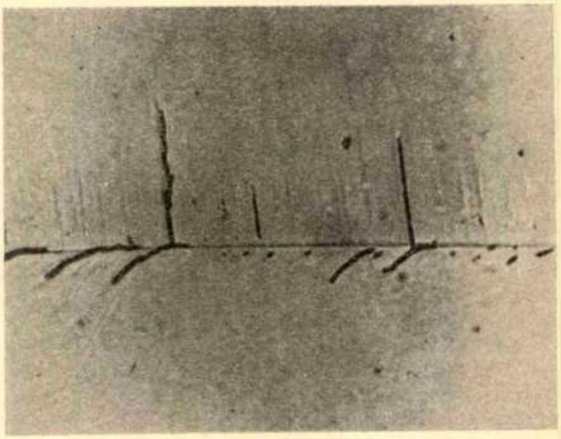

Fig. 3. Surface of fatigued beta-brass specimen. The same area as shown in Fig. 2 after extrusions and slip removed by electro-polishing. Persistent markings, that is, incipient cracks. (× c. 1,070)

One of the main observations has been the initiation of fatigue cracks and the formation of extrusions in short-slip markings, which form adjacent to grain boundaries, and in the majority of cases at an angle to the main slip. These cracks are afterwards propagated into and across the grain boundaries and frequently become linked together.

The most unusual features of this phenomenon are that these cracks form at an angle to the main slip but not in the main slip, and that they form adjacent to the grain boundaries but not in the grain boundaries (Figs. 1, 2 and 3).

Further work leading to a model for the mechanism is in progress.

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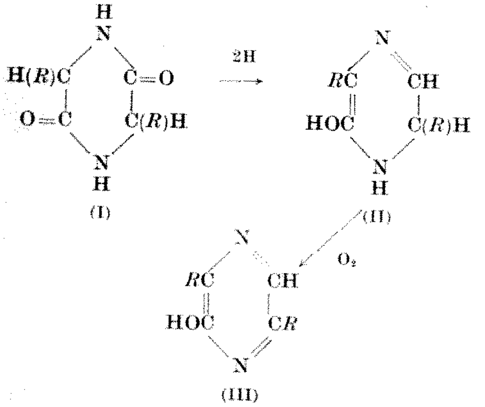
RADIATION CHEMISTRY

Production of Pyrazine Derivatives in the Radiolysis of Aqueous Solutions of the Cyclic Dipeptides

Radiation-induced reactions in aqueous solutions of amino-acids, peptides, and related compounds have been treated in a number of reports from this Laboratory[1]. In connexion with this work, we have had occasion to examine the effects of γ-irradiation on the ultra-violet absorption spectra of solutions of various simple peptides. The measurements reveal that solutions of the cyclic dipeptides under specific conditions exhibit pronounced and characteristic post-irradiation changes. If, for example, an oxygen-free solution of alanine anhydride is irradiated and examined spectrophotometrically, the differential spectrum (read against unirradiated solution) shows negligible absorption above 2600 Å. so long as the solution is not exposed to the atmosphere. However, if oxygen is introduced, an absorption maximum in the longer wave-length region slowly appears. This post-irradiation change is accelerated by addition of either acid or base. In neutral and basic solution the absorption maximum is at about 320 mμ; in acid solution the maximum shifts to 340 mμ. This shift is reversible, and the evidence is that the final yield of the absorbing species is essentially independent of the *p*H of the solution during the post-irradiation oxidation. Solutions of glycine anhydride exhibit quite similar properties except that the wave-length of maximum absorption (320 mμ) is independent of *p*H. It is also to be noted that these post-irradiation changes are not observed if oxygen is present during irradiation.

We believe the phenomenon involves the radiation-induced reduction (and subsequent dehydration) of the diketopiperazine molecule (I) to the corresponding 1 : 2-dihydropyrazine derivative (II) which is of a class of compounds known[2] to be readily oxidized (that is, in air) to the corresponding pyrazine structure (III)



Isolation of 2-hydroxy-3 : 6-dimethylpyrazine (III) from an irradiated solution of alanine anhydride (Nutritional Biochemicals Corporation, Lot 2104, recrystallized three times from water) has been effected. The irradiated solution was lyophilized, and an appropriate aliquot of a methanolic solution of the residue was transferred to a filter-paper strip, and then chromatographed in a butanol–ammonia solvent system[3]. At low radiation dosages a single fluorescent compound ($R_F \sim 0{\cdot}7$) was observed. This was eluted and compared spectrophotometrically with authentic material prepared according to the method of Baxter and Spring[4]. Fig. 1 shows a normalized plot of the results. Co-chromatographic studies of the authentic material and the isolated irradiation product fully support the identification. The initial yield of 2-hydroxy-3 : 6-dimethylpyrazine in 0·1 *M* alanine anhydride corresponds to a *G*-value of $\sim 0{\cdot}4$ (cobalt-60 γ-rays; 7×10^{19} eV./ml.).

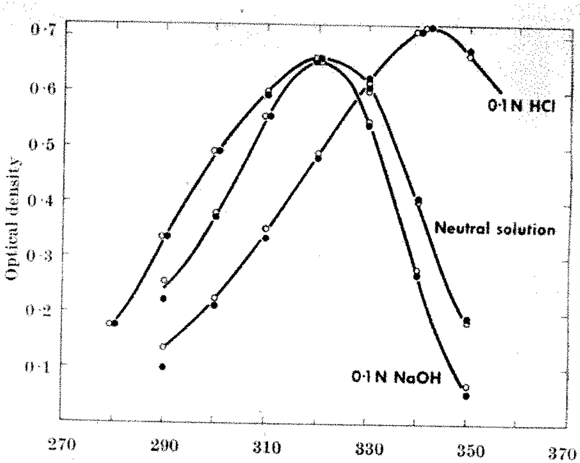


Fig. 1. Absorption spectra of authentic 2-hydroxy-3 : 6-di-methylpyrazine (○) and of product isolated from irradiated solutions of alanine anhydride (●)

This work was performed under the auspices of the U.S. Atomic Energy Commission.

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CHEMISTRY

Porous Glass Chromatography

Chromatographic separations have now been achieved using porous glass[1] as a medium. Its extreme inertness, optical transparency, and small pore diameter (about 4 mμ) suggested this usage. Initial results strongly indicate that unusually fine chromatograms can be obtained with this material. Other than in the choice of medium, there is little difference between the techniques described here and those commonly used in paper chromatography.

The present investigation has been concerned with the characterization of fountain-pen inks. The procedure is quite simple, consisting essentially of four steps. First, a 2-μl. sample of ink is placed about one-half of an inch from the edge of a small (3 in. × 3 in.) piece of porous glass. Secondly, the glass is exposed to the vapours of the liquid solvent to be used for development for 1 hr. Thirdly, the sample edge of the glass is dipped into the solvent almost up to the spot of ink and held in this position

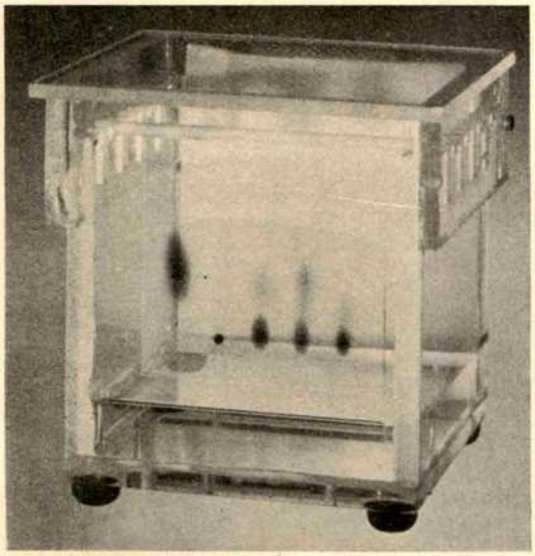

Fig. 1. Chromatographic cell

for about 24 hr. as shown in Fig. 1. Fourthly, and lastly, the glass is removed, wiped dry with a soft cloth, and placed in a warm place to remove completely the absorbed moisture.

Chromatograms resulting from this procedure have yielded excellent separation of the individual constituents depending on their relative solubilities, molecular size and shape, and relative retardation effect to the porous medium. Examples of chromatograms of five ink samples are illustrated in Fig. 2.

Unfortunately, a black-and-white reproduction of these porous glass chromatograms does not do justice to the colourful results which were actually obtained. Sample No. 41, for example, Carter's 986 black ink, resulted in a blue spot where the original sample was placed, a deep green over this, followed by orange and finally a rose or violet on top.

The other inks shown in Fig. 2 are identified: No. 38, Waterman's new permanent black; No. 39, Waterman's old permanent black; No. 42, Higgin's No. 812 eternal permanent black; No. 50, Carter's No. 99 permanent black.

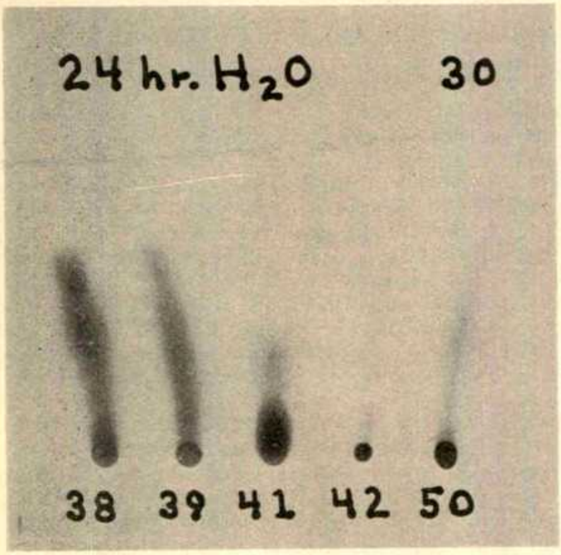

Fig. 2. Porous glass chromatogram

There are several advantages in the use of porous glass as compared to conventional paper for a chromatographic medium. Very high surface area of porous glass (about 150–200 m.^{2} per gm.) confines the sample to a small area due to immediate drying. This, in addition to longer development time, provides improved resolution or definition of the bands. Porous glass can be used with nearly all solvents except hydrofluoric acid and strong alkaline solutions. Being an inorganic inert material, organic contamination is at a minimum and pyrolysis rates of specific components have been observed as individual bands changing in appearance. Porous glass chromatograms can be permanently filed for reference. Optical measurements, including transmission, reflectance and fluorescence, have been made directly on the dry, transparent porous glass after the chromatographic separations have been accomplished.

The original interest in the use of porous glass as a chromatographic medium has been a result of my interest and activity in the field of criminology. It must be pointed out, however, that this medium is not in any way limited to the characterization of fountain-pen inks. The separations and/or purification of body fluids, virus, bacteria and other serological fluids which may be obtained due to their actual molecular dimensions must be investigated. Observations have been made which establish the fact that certain molecules are too large to pass through the small holes in porous glass. Finally, the use of this medium as a support for electrophoretic separations offers possibilities as yet unexplored.

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Two New Amino-Sugars from an Antigenic Polysaccharide of *Pneumococcus*

THOUGH a large number of antigenic capsular polysaccharides of *Pneumococcus* are known to contain one or more amino-sugars[1], in only one case, namely, 2-amino-2-deoxy-D-glucose (D-glucosamine) from type XIV polysaccharide[2], has the amino-sugar been isolated and characterized. Two amino-sugars, designated 'pneumosamine' and 'amino-sugar X', have been isolated from purified Type V *Pneumococcus* capsular polysaccharide[3], and preliminary investigations, which included analysis of the crystalline *N*-acetamido alcohol derivatives, indicated that both sugars were 2-amino-2,6-dideoxyhexoses. Kuhn-Roth determinations on '*N*-acetylpneumosaminol' (1·52 moles CH_3) and on 'amino-sugar X' (0·68 mole CH_3) support the postulated 6-deoxyhexosamine structure. The sugars have now been tentatively identified as 2-amino-2,6-dideoxytalose (pneumosamine) and 2-amino-2,6-dideoxy-L-galactose (amino-sugar X). The sugars were isolated from a polysaccharide hydrolysate (hydrolysis conditions: 2 *N* hydrochloric acid, 24 hr., 100°) by chromatography on 'Dowex-50' resin[4]. The acidic effluents were neutralized with 'Deacidite *FF*' (carbonate form), lyophilized and the syrupy residues dried *in vacuo* over phosphorus pentoxide. Treatment of the 'pneumosamine' residue with methanol gave 'pneumosamine hydrochloride' melting point 162–3° (from methanol–ether); $[\alpha]_D^{18} + 6\cdot9^\circ$ (10 min.), $\rightarrow + 10\cdot4^\circ$

equil. (conc. 2·3 in water). Found: C, 35·9; H, 7·4 per cent; calc. for $C_6H_{14}O_4NCl$: C, 36·1; H, 7·1 per cent. 'Amino-sugar X' was crystallized from a water–methanol–acetone mixture.

A comparison of the electrophoretic mobilities of the *N*-acetyl derivatives of 'pneumosamine' (M_G 0·40), 2-amino-2,6-dideoxy-L-galactose (M_G 0·29) and 2-amino-2,6-dideoxy-D-glucose (M_G 0·0) in borate buffer indicated that the hydroxyl groups on $C_{(3)}$ and $C_{(4)}$ were *cis* related, since vicinal *trans* hydroxyl groups (as in 2-acetamido-2,6-dideoxy-D-glucose) do not complex with borate[5,6]. Such *cis*-related hydroxyl groups are present in sugars having the *allo*-, *altro*-, *galacto*- and *talo*- configurations. 'Pneumosamine' and 2-amino-2,6-dideoxy-L- or -D-galactose had different paper and ion-exchange chromatographic properties and optical rotations. The best method of distinguishing between the remaining three configurations seemed to be by deamination, despite the complexity of the reaction[7,8]. A chromatographically homogeneous product was obtained from 'pneumosamine' by deamination under rigorously controlled conditions. This product migrated the same distance as 6-deoxy-L-galactose and separated on the same chromatogram from 6-deoxyallose, 6-deoxyaltrose, 6-deoxytalose and 6-deoxymannose. The deamination therefore indicated that 'pneumosamine' was probably 2-amino-2,6-dideoxytalose, and that deamination proceeded with inversion at $C_{(2)}$. An analogy may be drawn with the deamination of 2-amino-2-deoxy-D-mannose (axial amino group), which proceeds with Walden inversion at $C_{(2)}$ (refs. 9 and 10). Deamination of 2-amino-2-deoxy-D-glucose (equatorial amino group) proceeds with ring contraction and the product is an anhydro sugar[9,11]. Possibly the difference in energy between the two chair conformations of 2-amino-2,6-dideoxytalose is sufficient, under the conditions of deamination, for the conformation with an axial amino group to predominate.

'Amino-sugar X' and its *N*-acetyl derivative were found to be indistinguishable from 2-amino-2,6-dideoxy-L-galactose (L-fucosamine) and 2-acetamido-2,6-dideoxy-L-galactose by paper and ion-exchange chromatography and by electrophoresis. The infra-red spectra of an equilibrium mixture of the isolated sugar and authentic L-focusamine were almost completely superimposable. *N*-Acetylation of a crude sample of 'amino-sugar X', followed by separation on Whatman No. 3 filter paper, gave a crystallizable product of melting point 195–198° (uncorr.), undepressed on admixture with authentic *N*-acetyl-L-fucosamine, $[\alpha]_D^{20}$ − 79° equil. (conc. 0·17 in water). The infra-red spectra of an equilibrium mixture of the *N*-acetylated product and of authentic *N*-acetyl-L-fucosamine were also identical. The amino-sugar was assigned to the L-series on the basis of optical rotation data. Furthermore, it was possible to epimerize[12] '*N*-acetylpneumosamine' to an equilibrium mixture containing a product chromatographically indistinguishable from either authentic *N*-acetylfucosamine or '*N*-acetylamino-sugar X'. This affords additional evidence that 'pneumosamine' and 'amino-sugar X' are epimeric at $C_{(2)}$.

Neither 2-amino-2,6-dideoxy-L-galactose (L-fucosamine) nor 2-amino-2,6-dideoxytalose has been previously found in Nature, and the latter sugar was hitherto unknown. Its synthesis is at present being undertaken in our laboratories. The discovery of these amino-sugars in the amino-sugar – rich *Pneumococcus* species should stimulate much work, both chemical and immunological, in order to improve our understanding of the specificity of immunological reactions.

We are indebted to Profs. R. Kuhn and T. Reichstein, Drs. D. A. L. Davies and C. J. Morel for providing gifts of amino-sugars and 6-deoxyhexoses. We are also indebted to Prof. M. Heidelberger, who supplied the Type V polysaccharide. Two of us (M. J. H. and J. M. W.) thank the University of Birmingham for research grants.

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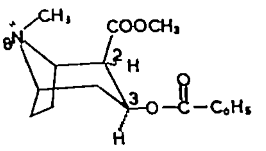
Specification of Asymmetric Nitrogen Atom. Application of the Cahn–Ingold–Prelog Nomenclature to some Tertiary Amines and Quaternary Ammonium Salts

THOUGH the Cahn–Ingold–Prelog nomenclature[1] is quite generally valid for specifying asymmetric systems, and was proposed by its authors for the unambiguous description of both organic and inorganic systems, little application is to be found in the literature[2] so far relating to the characterization of nitrogen atoms of known configuration by this convention.

The specification of an asymmetric nitrogen atom follows from the general definition given by Cahn, Ingold and Prelog. Accordingly, in the case of a tertiary nitrogen atom, the three substituents attached to it should be regarded as the *a*, *b* and *c* groups, whereas the fourth substituent (*d*) of zero ordinal number is the unshared electron pair. Existence of tertiary amines of stable configuration makes it necessary to accept the orientation of the unshared electron pair of the nitrogen atom; this fact can be deduced both from quantum mechanical calculations[3] and evidence obtained in a synthetical chemical way[4].

Compounds of known configuration and containing an asymmetric tertiary nitrogen atom are known among the tropane alkaloids[5]. Fodor *et al.*[6] showed that in the case of the tertiary tropane derivatives investigated, the substituents attached to the nitrogen and not contained within the ring had N-α-configurations. Summarizing the results of the investigations



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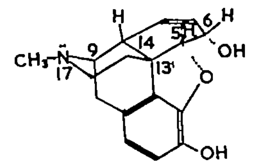

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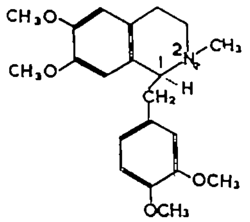

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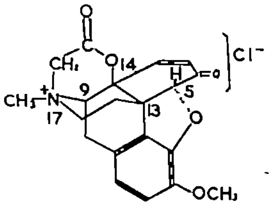

IV

of Hardegger and Ott[7] relating to the absolute configuration of the C_2 and C_3 carbon atoms of (−)-cocaine, and those of Kovács, Fodor and Halmos[8] as regards the absolute configuration of the asymmetric nitrogen atom of the skeleton, natural (−)-cocaine (I) may be described as having a 2*R* : 3*S* : 8*R* configuration.

In the course of earlier research work we[9] reported the configurational stability of the tertiary nitrogen atom of the morphine skeleton. Assuming an equatorial position of the N-methyl group (which is also supported by our recent investigations[10]) the asymmetric tertiary nitrogen atom will be of *S*-configuration. Consequently, morphine (II) should be regarded as having 5*R* : 6*S* : 9*R* : 13*S* : 14*R* : 17*S* configuration[11].

It is known from the work of Corrodi and Hardegger[12] that the C_1 atom of (+)-laudanosine (III) has the *S*-configuration. Since Knabe[13] ascribes an equatorial steric position for the C_1 substituent of laudanosine and an axial position for the N-methyl group, it follows that the nitrogen is of *R*-configuration.

The assignment of asymmetric quaternary ammonium salts is obvious. It follows that 14-hydroxycodeinone lactone hydrochloride[10] (IV) may be characterized by the configuration 5*R* : 9*R* : 13*S* : 14*R* : 17*S*.

Application of the Cahn–Ingold–Prelog nomenclature, in the sense shown here, to tertiary amines having nitrogen atoms of known configuration and of configurational stability, as well as to quaternary ammonium salts, permits a more complete and unequivocal description of the compounds.

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BIOCHEMISTRY

Non-Enzymic Decarboxylation of Methionine by Ferrous Ion

IN work on the precursors of the volatile sulphur components of vegetable flavours and odours, a non-enzymic oxidative decarboxylation of methionine in the presence of Fe^{2+} was observed. The final reaction mixture had a very pronounced odour. Unlike other well-known Fe^{2+} oxidations, there was no need for added hydrogen peroxide[1,2] or ascorbic acid[3,4]. The addition of a metal chelating agent such as ethylenediamine tetraacetate (EDTA) or citrate was very stimulatory. Table 1 shows the absolute requirement for Fe^{2+}. Only Fe^{2+} was able to carry out this decarboxylation. Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Al^{3+} were completely ineffective. Fe^{3+} was 1 per cent as effective as Fe^{2+}.

Table 1. REQUIREMENTS FOR METHIONINE DECARBOXYLATION

Reaction system*	mμc. ^{14}C as CO_2
Complete	58·8
Minus Fe^{2+}	0
Minus EDTA	13 1
Complete, 0·5 μmole Fe^{2+} instead of 5 μmoles	7·2

* Complete reaction system contained 2 μmoles 1-^{14}C DL-methionine containing 0·5 μc., 5 μmoles ferrous chloride, 5 μmoles EDTA, 100 μmoles phosphate buffer *p*H 7, and distilled water to a final volume of 2 ml. This solution was in the main chamber of a Warburg vessel. In the centre well was 0·2 ml. 20 per cent potassium hydroxide and in the sidearm 0·8 ml. 5 *N* sulphuric acid. The reaction was incubated 90 min. at 30° C., the acid tipped and the potassium hydroxide collected 10 min. later. The absorbed carbon dioxide was counted as a gas in an ionization-type counter.

The participation of free hydrogen peroxide did not seem to be indicated since the addition of crystalline catalase only inhibited the reaction by 19 per cent. The reaction was found to be aerobic in nature. In a nitrogen atmosphere the amount of carbon dioxide produced from the methionine carboxyl group was decreased by 88 per cent. The residual activity remaining in the nitrogen-flushed reaction mixture was probably due to the small amount of dissolved oxygen which had not been displaced by the method of flushing used.

The addition of ascorbic acid to the reaction mixture gave a large increase in the amount of carbon dioxide released. In the presence of ascorbic acid, Fe^{3+} was as effective as Fe^{2+}. EDTA was required for the stimulatory effect of ascorbic acid to be demonstrated. These results are summarized in Table 2.

Table 2. ASCORBATE EFFECT ON DECARBOXYLATION

Reaction system*	Total counts as $Ba^{14}CO_3$
Complete	23,990
Minus EDTA	7,255
Plus ascorbate	42,145
Plus ascorbate, minus EDTA	8,834
Complete, Fe^{3+} for Fe^{2+}	332
,, ,, ,, ,, plus ascorbate	42,885
,, ,, ,, ,, ,, ,, minus EDTA	8,804

* Reaction system and conditions same as in Table 1. 5 μmoles ferric chloride and 10 μmoles ascorbic acid are added where indicated. Absorbed $^{14}CO_2$ was precipitated, plated and counted as $Ba^{14}CO_3$ by a thin-window Geiger–Müller-type counter.

The question whether the ascorbic acid effect is due to maintenance of Fe^{2+} concentration or to a different mechanism is left open. Preliminary work indicates that the reaction is not of an important general nature as regards amino-acids. 1-^{14}C DL-alanine was decarboxylated to only 1 per cent and 1-^{14}C DL-methionine sulphoxide to only 6 per cent that of an equivalent amount of methionine under the same conditions.

A large-scale reaction was carried out in order to isolate the reaction product. 250 µmoles phosphate buffer *p*H 7, 200 µmoles ferrous chloride, 200 µmoles EDTA *p*H 7, and 500 µmoles DL-methionine in a final volume of 9·5 ml. were incubated for 90 min. at room temperature with constant shaking. The reaction mixture was then acidified with 3 ml. 10 per cent trichloracetic acid, and 10 ml. 0·01 *M* 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid were added. After cooling at 10° C. overnight, the precipitate which formed was removed by filtration and after drying over calcium chloride was recrystallized from ethanol–water. An infra-red spectrum of a potassium bromide pellet of the recrystallized material was obtained from 3 to 15µ. This spectrum was identical with that of a known sample of the 2,4-dinitrophenylhydrazone of methional. It would appear that the decarboxylation is also accompanied by some deamination to yield the corresponding aldehyde.

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Chromatography of the Proteins from Wheat Flour soluble in Acetic Acid

THE heterogeneity of the gluten fraction of wheat flour has been well established by the moving boundary[1–3], starch-gel[4] and filter paper curtain[5] methods of electrophoresis. The last-mentioned technique has also been used for fractionation purposes. The chromatographic separation of gluten into four major electrophoretically distinguishable components by stepwise elution from carboxymethyl-cellulose has also been reported briefly[1,6]. This communication reports the resolution of the acetic-acid-soluble fraction of flour into six components by gradient elution from carboxymethyl-cellulose.

Flour, milled from Gabo wheat to approximately 70 per cent extraction on a Buhler laboratory mill, was extracted successively with water-saturated *n*-butanol (1 treatment), 0·01 *M* sodium pyrophosphate (*p*H 7·0 ; 4 treatments) and 0·05 *N* acetic acid (*p*H approximately 3·5 ; 4 treatments) in accordance with the procedure described by Coates and Simmonds[7]. Under these conditions 18–20 per cent of the nitrogen was soluble in the sodium pyrophosphate, 55–60 per cent was soluble in the acetic acid and the remainder could be extracted with 0·1 *N* sodium hydroxide. The butanol extract contained only 0·5–2·0 per cent of the total nitrogen.

The acetic acid extract, containing 50–100 mgm. of protein nitrogen in 100 ml., was dialysed against 0·005 *M* sodium acetate buffer *p*H 4·1, and loaded on to a carboxymethyl-cellulose column (1·5 × 15 cm.) previously equilibrated with the same buffer. Under these conditions most of the protein was bound to the carboxymethyl-cellulose, but a small amount, designated fraction α, passed unretarded through the column. In order to remove completely the remainder of fraction α, the column was washed with a further 30 ml. of the 0·005 *M* sodium acetate buffer, followed by 300 ml. of the same buffer containing 1·0 mole of dimethyl-formamide per litre (final *p*H 4·1). Three protein components (peaks β, γ and δ in Fig. 1) were then eluted using a sodium chloride gradient running from 0 to 1 *M*, total volume 1 l., the molar concentrations of sodium acetate and dimethyl-formamide being maintained at 0·005 and 1·0 respectively. Two additional components (peaks ε and θ in Fig. 1) could be eluted by running a further gradient from acetate buffer 0·005 *M* in sodium acetate (*p*H 4·1) to a phosphate solution *p*H 12, containing 0·005 mole of trisodium phosphate per litre, both solutions being 1·0 *M* with respect to sodium chloride and dimethyl-formamide : the total volume of this gradient was also one litre. Any protein still adhering to the column was then removed with 0·1 *M* sodium hydroxide (peak ω in Fig. 1) : this peak is not considered to represent a definite protein component and has been included in the elution diagram to indicate the amount of protein not recovered from the column by the two gradients employed. Fig. 1 shows the sequence of the elution pattern and the *p*H of the effluent at each stage, together with an estimate of the protein distribution between peaks, determined by the method of Lowry, Rosebrough, Farr and Randall[8].

Using this procedure, a series of acetic acid extracts from 12 Australian flours was examined, 100 mgm. of protein nitrogen being loaded in each case. This allowed a resolution of the main protein components similar to that shown in Fig. 1. However, the solubilities of these were such that material in the peak tubes of components β, δ, ε and θ was precipitated on elution from the column. This precipitate was soluble in 0·05 *M* acetic acid. The only obvious difference in the elution patterns obtained was a variation in the size of the δ peak, which was not even detected in several cases.

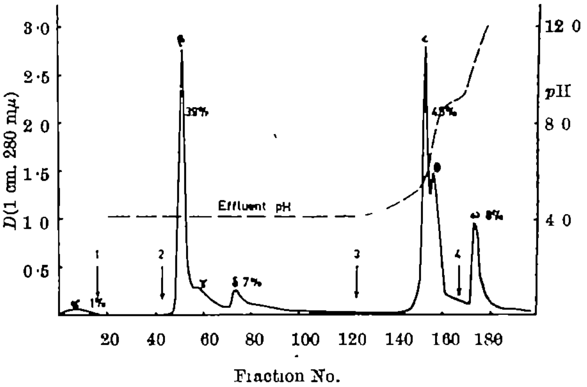

Fig. 1. Elution of flour protein components from a carboxymethyl-cellulose column. Loading : 100 mgm. of protein nitrogen derived from the 0·05 *M* acetic acid soluble fraction of Gabo flour Column : 1·5 cm. × 15 cm., equilibrated to *p*H 4 1 with 0 005 *M* sodium acetate buffer. Size of fractions . 12 ml. Eluting solvents changed as follows . Arrow No. 1, 0 005 *M* sodium acetate containing 1 0 *M* dimethyl formamide ; *p*H 4 1. Arrow No. 2, gradient started to 0 005 *M* sodium acetate containing 1 0 *M* dimethyl formamide and 1·0 *M* sodium chloride ; *p*H 4·1. Arrow No. 3, gradient started from previous final solvent to 0 005 *M* trisodium phosphate containing 1·0 *M* dimethyl formamide and 1 0 *M* sodium chloride ; *p*H 12·0. Arrow No. 4, 0·1 *N* sodium hydroxide

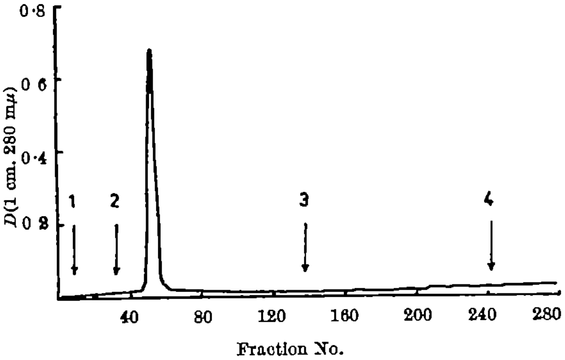

Fig. 2. Rechromatography of fraction β (Gabo flour) on carboxymethyl-cellulose. Loading: 15 mgm. of protein nitrogen. Column: 1·5 cm. × 15 cm., equilibrated to pH 4·1 with 0 005 M sodium acetate buffer. Size of fractions: 10 ml Order of change in eluting solvents as indicated in Fig. 1

Rechromatography of fraction β on carboxymethyl-cellulose gave a symmetrical elution peak as shown in Fig. 2, but there is no certainty that this or any of the peaks would behave as a single homogeneous protein when subjected to other methods of fractionation. In fact, Elton and Ewart[4] have demonstrated by starch-gel electrophoresis the presence of at least eight components in acetic acid extracts of glutens obtained by hand-washing flours with distilled water. It is possible that some of these components correspond to traces of albumin and globulin fractions[9], which would have been removed during our preliminary treatment of the flour with sodium pyrophosphate.

Dimethyl formamide, which has been used by Vercouteren and Lontie[10] to disperse gluten, was added to the buffer to prevent precipitation of protein from solutions of ionic strength greater than 0·04. In this case it has allowed the successful elution of components from the column using salt and mild pH gradients. There is evidence that the reaction between hydrogen bond - breaking agents and gluten is reversible[11,12].

Full details of this work will be submitted for publication in due course.

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Optical Rotatory Dispersion of the Thrombin-induced Conversion of Fibrinogen to Fibrin Monomer

CONSIDERABLE evidence has now accumulated suggesting that the thrombin-induced conversion of fibrinogen to fibrin is a reversible triphasic reaction which may be represented thus[1]:

Step 1 Proteolysis $F \overset{T}{\rightleftarrows} f + P$
Step 2 Polymerization $nf \rightleftarrows f_n$
Step 3 Clotting $mf_n \rightleftarrows$ fibrin

where F is fibrinogen, T is thrombin, f is fibrin monomer, P represents non-protein nitrogenous material consisting primarily of two acidic peptides of molecular weights 1,900 and 2,400, and f_n represents intermediate polymers where n and m are variable numbers.

Hydrogen bonding has been implicated in this reaction scheme in two important aspects. First, the polymerization of f to intermediate polymers (step 2) has been shown to proceed through the formation of intermolecular hydrogen bonds between tyrosyl donors and histidyl acceptors[2]. Secondly, it has been suggested that either the donor or acceptor groups are unavailable in the intact fibrinogen molecule because they are hydrogen-bonded to the highly acidic groups of the two peptides released in step 1; they become available only when the hydrogen bonds in which they are participants are broken[1]. If such hydrogen bonds exist in the native fibrinogen molecule, they should be playing a significant part in stabilizing that portion of the molecule which is regularly folded; conversely, the rupture of these bonds should decrease the stability of the helical portions of the molecule. In order to observe whether configurational changes of this type accompany the thrombin-catalysed proteolysis of fibrinogen, measurements of the optical rotatory dispersion of this system were undertaken. To facilitate studies of step 1 free of steps 2 and 3, use was made of the finding that step 1 will occur in 1 M sodium bromide at pH 5·3 without the subsequent polymerization of fibrin monomer[3].

The bovine fibrinogen used ('Pentex' lot 20F 14) was refractionated by Laki's procedure[4]; a product which was 95 per cent clottable, as assayed by the method of Laki[4], was obtained. A purified citrate-activated thrombin preparation kindly supplied by Dr. W. H. Seegers was used. This product, received and stored in aqueous solution, had an activity of 4,000 tosylarginine methyl ester units/mgm. The optical rotatory measurements were carried out on a Rudolph model 260 automatic recording spectropolarimeter using a xenon compact arc-lamp as the light source. Kinetic measurements were made at 400 and 589 mμ. At the beginning and end of each reaction a complete optical rotatory dispersion curve was run; in these cases the results were evaluated in accordance with the method of Yang and Doty[5], by plotting the product of the specific rotation and squared wave-length versus specific rotation.

Column 2 of Table 1 lists the variation of optical rotation with wave-length for a 2·5 per cent fibrinogen solution in 1 M sodium bromide at pH 5·3 (0 time curve). It is to be noted that $[\alpha]_D$ is $-$ 53° and λ_0, evaluated from the slope of the plot of $\lambda^2[\alpha]$ versus $[\alpha]$, is 227 mμ; these values are to be compared with an $[\alpha]_D$ of $-$ 48·6° and a λ_0 value of 256 mμ, obtained

Table 1. OPTICAL ROTATORY DISPERSION RESULTS FOR FIBRINOGEN AND FIBRIN MONOMER

Wave-length, λ (Å.)	$-[\alpha]$ for fibrinogen (2·63 per cent)	$-[\alpha]$ for fibrin monomer (2 55 per cent) formed from fibrinogen of column 2	$-[\alpha]$ for fibrin monomer (3·2 per cent) formed from fibrin
5890	53	51	53
5500	65	63	62
5000	84	80	81
4500	103	99	100
4250	122	118	116
4000	141	141	141
3800	171	165	162
3700	—	—	181
3600	201	200	197
3500	224	223	216
3400	257	247	—
3300	285	286	—
3200	327	325	—
λ_0 (mμ)	226·9	228 0	225·8

by Yang and Doty for fibrinogen in 0·3 *M* potassium chloride at *p*H 6·3[5]. The increase in lævorotation and decrease in dispersion constant in the bromide solvent are consistent with the idea that this medium is a denaturing one[6] and that it prevents steps 2 and 3 from setting in because of its ability to break hydrogen bonds.

At zero time, thrombin was added to the fibrinogen solution in the polarimeter cell such that its final concentration was 100 units/ml. The cell contents were mixed and the rotations at 400 and 589 mμ were followed for 20 hr. (half-time about 3 hr. at 25° C.[7]): these were not found to vary significantly, suggesting that the kinetics of the reaction are not reflected in optical rotatory changes. In this respect, this reaction differs from other examples of limited proteolysis reactions, notably the zymogenic activation reactions of chymotrypsinogen, trypsinogen and pepsinogen, all of which are accompanied by easily measurable changes in optical rotation[8]. At the completion of the reaction the solution was exhaustively dialysed against 1 *M* sodium bromide at *p*H 5·3 in order to remove the liberated peptides and an optical rotatory dispersion curve was carried out on the remaining fibrin monomer (see column 3 of Table 1). As can be seen, none of the rotations for the fibrin monomer differs significantly from the values obtained for fibrinogen alone; furthermore the calculated λ_0 values agree within experimental error, suggesting that the degree of ordered configuration in the two molecules is the same. As a further check that the dispersion results of column 3 represent the values for fibrin monomer and not those of monomer plus adsorbed contaminants, purified fibrin monomer was obtained by dissolving fibrin in 1 *M* sodium bromide at *p*H 5·3 (that is, by reversing steps 2 and 3 in the bromide solvent[3]). The optical rotatory dispersion of a 3·2 per cent solution of this material is presented in the final column, from which it is seen that essentially identical optical rotatory results are obtained for fibrin monomer prepared by either the forward or backward reactions.

The virtual identity in optical rotatory properties of native fibrinogen and fibrin monomer suggests that the two peptides liberated in the proteolysis reaction must be essentially devoid of helix; otherwise their removal would have resulted in different dispersion constants for the two solutions. This finding is consistent with the preliminary amino-acid analysis of the two peptides[9], which reveals in each the presence of a number of dicarboxylic amino-acid residues as well as two prolines; both the high density of negative charge and presence of two imino-acids would tend to favour a randomly oriented configuration. Finally, the lack of optical rotatory change associated with the kinetic process implies that the donor or acceptor groups liberated in step 1 appear, more likely as the result of an unmasking process, that is, a removal of a steric impediment exerted by the peptides rather than by rupture of side-chain hydrogen bonds which would be expected to have been reflected in optical rotatory changes.

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The Problem of 'Methæmocyanin'

IT was thought that, analogously to the iron in hæmoglobin, the copper of hæmocyanin remained in the cuprous (Cu^+) state when the pigment took up oxygen[1]. This was questioned[2] because the characteristic blue colour of the cupric ion (Cu^{++}) appeared when oxyhæmocyanin was formed, as well as on other grounds. It has since been shown[3], using the hæmocyanin of *Busycon*, *Loligo*, *Homarus* and *Limulus*, that in fact half the copper becomes Cu^{++} and the rest remains Cu^+. There was occasion to confirm this on the bloods of *Helix* and *Maia*, in the course of other work[4]. Klotz and Klotz[3] showed that the oxygenation probably depends on the co-operation of the two ions. A number of further differences in properties between hæmocyanin and hæmoglobin also were explained by their important discovery.

There still remains some obscurity, however, concerning 'methæmocyanin', which is prepared, like methæmoglobin, by the action of strong oxidizing agents on the pigment[5]. It is not clear why methæmocyanin should still be able to take up oxygen reversibly, with a colour change essentially similar to that of hæmocyanin itself. Since half the copper is converted to Cu^{++} even by atmospheric oxygen it appears to be more easily oxidized than the iron of hæmoglobin. Yet the latter is entirely converted to ferric in methæmoglobin and can no longer take up oxygen reversibly. It has been confirmed, using *Maia* blood, that hæmocyanin does retain this ability for reversible oxygenation–deoxygenation, with colour change, in the presence of such strong oxidizing agents as potassium ferricyanide and potassium permanganate. If the oxidizing agent is added to two samples of deoxyhæmocyanin and one is then shaken with air, the change in colour is readily apparent by comparison with the control.

It is also clear that oxidizing agents do not increase the amount of blue Cu^{++} ion present in oxyhæmocyanin or produce any in deoxyhæmocyanin. There is no increase in extinction of light of 570 mμ wavelength when *Maia* blood is saturated with potassium chlorate or treated with other colourless oxidizing agents, such as potassium iodate and hydrogen peroxide (neutralized with sodium peroxide). This may be confirmed also using potassium ferricyanide which absorbs very little light at that wave-length. The evidence is that the copper is quite 'unavailable' to these and also to other agents, though available to atmospheric oxygen. For example, sodium diethyldithio-carbamide, a sensitive, specific reagent for copper ions, gives no colour reaction with native *Maia* blood, though this appears sharply at a certain stage of denaturation, for example, by heating to 60° C. This applies equally to the reaction with 2 : 2′-diquinolyl, a specific colour reaction of the Cu^{+} ion[3], alone.

If an excess of such oxidizing agents as potassium ferricyanide and potassium permanganate is added to *Maia* blood, which is then denatured to 'reveal' the copper ions, it is found that all are now in the Cu^{++} state. Oxidation of free Cu^{+} to Cu^{++} is presumable instantaneous, as shown also by adding the oxidizing agent after denaturation in the presence of diquinolyl, in a suitable solvent (glacial acetic acid is most convenient since it also denatures the protein, and redissolves it on warming). Using potassium chlorate as oxidizing agent, some of the Cu^{+} survives for a time after release by denaturation and the pink colour with diquinolyl fades slowly.

A further feature of interest is that the Cu^{++} of denatured hæmocyanin oxidized by potassium permanganate returns spontaneously to Cu^{+}, slowly at room temperature and more rapidly on warming. Preparations from oxyhæmocyanin give a final yield of Cu^{+} about half the amount obtained from deoxyhæmocyanin preparations, indicating that ions initially in the Cu^{++} state remain so. However, on repeated oxidation with potassium permanganate, followed by spontaneous reduction, the amount of Cu^{+} progressively increases. Reducing agents still readily reduce the Cu^{++} to Cu^{+}. Potassium dichromate shows the same behaviour as potassium permanganate ; but ferricyanide, chlorate and hydrogen peroxide, in excess, appear to oxidize irreversibly. Using minimal amounts of hydrogen peroxide the ion can be reduced once more simply by exposure to low oxygen tension, over alkaline pyrogallol, so that denatured hæmocyanin might still be a reversible oxygen carrier, under appropriate conditions.

It is interesting that potassium permanganate should have been used[5] to prepare 'methæmocyanin', rather than ferricyanide, which is used for methæmoglobin and seems more capable of effecting irreversible change. The reason given was that ferricyanide does not release oxygen from oxyhæmocyanin, though there seems no good evidence that permanganate does. In any event, however, neither seems to affect the copper of the native pigment. This seems much more sensitive to reducing than to oxidizing agents therefore.

Some of these properties of hæmocyanin are mimicked by synthetic copper proteins, a further indication that there is no special prosthetic group in hæmocyanin. When ten volumes of 1 per cent egg albumen solution are added to one of 0·1 per cent copper sulphate, a copper protein is formed, the colour of the Cu^{++} ion becoming intensified. Moreover, on adding diquinolyl in *iso*amyl alcohol a positive reaction for the Cu^{+} ion gradually develops. The protein is near its isoelectric point and denatures spontaneously. At a more alkaline *p*H it can be denatured by heat without release of the Cu^{+} ion which, however, appears on acidification. The albumen appears to bind and to reduce some of the copper. It is even capable of reducing Cu^{++} added after it has been denatured with acetic acid. The Cu^{+} of the denatured copper protein, in acetic, is oxidized by potassium permanganate and spontaneously reduces on standing. This is also true of the copper protein of *Lithobius*[4], in the reduced, denatured state.

Thus it seems that proteins commonly have the power to reduce Cu^{++} to Cu^{+} and to do so repeatedly following reoxidation, whether it is bound in non-ionic form to the native protein or associated as an ion, after denaturation. In native hæmocyanin, at least, the $Cu^{+} \rightleftharpoons Cu^{++}$ change is associated with reversible oxygenation, and this process is not affected by strong chemical oxidizing agents. It would seem reasonable to keep the term 'methæmocyanin' in abeyance until there is evidence of a definite compound similar to methæmoglobin.

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Activation of Cockroach Acetylcholinesterase by Water-miscible Organic Solvents

IT is common practice to treat insects with poisons dissolved in acetone or dioxane. Also, water-insoluble anti-cholinesterases are dissolved in organic solvents to determine their inhibition of acetylcholinesterase *in vitro*. While studying functional properties of acetylcholinesterase of the American cockroach[1], evidence was obtained that inclusion of acetone or some alcohols in a Warburg reaction mixture resulted in greater hydrolysis of acetylcholine. These results are given in Table 1, where 0·1 ml. of the organic solvents was included in a Warburg reaction mixture of salts, buffer, substrate and tissue homogenate, final volume, 2·7 ml. The increase in hydrolysis of acetylcholine was most striking in the presence of *n*-propanol and *n*-butanol. Further work showed that the optimal concentration of *n*-butanol for maximal hydrolysis of acetylcholine was 0·05 ml. in 2·7 ml. of reaction mixture. Acetone differed in this respect and optimal activity was obtained at 0·25 ml. of acetone in 2·7 ml. of reaction mixture.

Bovine purified erythrocyte and homogenates of housefly thoracic ganglia and mouse brain were used as sources of acetylcholinesterase for a comparison

Table 1. Effect of Various Solvents* on the Rate of Hydrolysis of Acetylcholine by Acetylcholinesterase from Cockroach Ganglia

Solvent	Average b_{30}†	Activation ratio $\frac{\text{Solvent } b_{30}}{\text{Control } b_{30}}$
iso-Octane	48·0	1·05
Dioxane	48·1	1·05
Ethyl alcohol	50·7	1·1
Acetone	71·5	1·56
iso-Butanol	106·0	2·31
iso-Amyl alcohol	124·5	2·72
iso-Propanol	132·1	2·89
n-Propanol	142·7	3·12
n-Butanol	180·8	3·95
Control	45·7 ± 2·1	

* 0·1 ml. acetone in 2·7 ml. of reaction mixture.

† b_{30} refers to the amount of carbon dioxide evolved in 30 min. after correction for non-enzymatic hydrolysis.

with homogenates of the thoracic nerve cord of the cockroach in the presence of solvents. The results given in Table 2 for acetone show that only the acetylcholinesterase of the cockroach was activated. The acetylcholinesterase from the other sources was inhibited when compared with controls in which acetone was omitted. This result shows a striking difference in the acetylcholinesterase of the cockroach-hydrolysing acetylcholine.

Table 2. Effect of Acetone* on Acetylcholinesterases from Various Sources

Source	Average b_{30}† control	Average b_{30} with acetone	Activation ratio $\frac{\text{Acetone } b_{30}}{\text{Control } b_{30}}$
Cockroach thoracic ganglia	48·2	83·1	1·72
Housefly thoracic ganglia	37·4	31·3	—
Mouse brain	140·4	115·8	—
Bovine purified erythrocyte	225·4	183·0	—

* 0·1 ml. acetone in 2·7 ml. of reaction mixture.

† b_{30} refers to the amount of carbon dioxide evolved in 30 min. after correction for non-enzymatic hydrolysis.

As the above results were determined with homogenates, experiments were then carried out with acetone and butanol powders of the nervous tissue of the cockroach. Manometric determinations of the reconstituted acetylcholinesterase showed that the activation effect of *n*-butanol and acetone was not eliminated when these solvents were included in the reaction mixture. Furthermore, these results showed that the effect found with homogenates was not due to a liberation of more acetylcholinesterase from tissue fragments or to an inhibiting factor. The use of differential centrifugation and various substrates gave positive evidence that only one enzyme hydrolysed acetylcholine. The enzyme appears to be particulate, and when solubilized by use of sodium cholate similar activation was found with *n*-butanol.

Results obtained on the kinetics of hydrolysis of acetylcholine and other substrates in the presence of *n*-butanol and anticholinesterases gave evidence of the possible mechanism of activation. This appears to be an effect of the solvents on the active sites of the acetylcholinesterase of the cockroach. If so, a specific property has been found for the acetylcholinesterase of the nervous tissue of the cockroach. Details of these results will be published elsewhere.

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O-Demethylation as a Biosynthetic Pattern in the Formation of Opium Alkaloids

Comparative rates of incorporation of carbon dioxide labelled with carbon-14 led to the proposal[1] that, in *Papaver somniferum*, thebaine is the first octahydrophenanthrene alkaloid formed, and is converted by successive O-demethylations to codeine and finally to morphine. Although this sequence seemed best to explain the labelling ratio among these three alkaloids, conclusive proof was lacking. The results described here, considered with the previous data[1], constitute proof of this interrelationship.

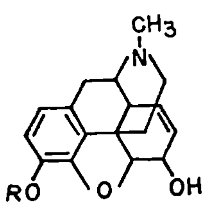
Three plants of *P. somniferum*, each 2½ months old, were root-fed, respectively, labelled morphine, codeine and thebaine, which were obtained from a three-day biosynthesis with labelled carbon dioxide[1]. The alkaloids were absorbed from a weak potassium dihydrogen phosphate solution over the course of 42 hr., and the plants then were frozen immediately and the alkaloids isolated as previously described[1]. The results are given in Table 1.

Table 1. Carbon-14 labelled Alkaloid Feeding Experiments

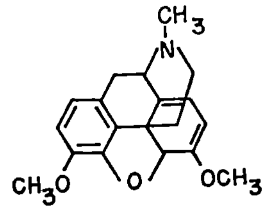
Plant No.	Alkaloid fed	Alkaloid isolated	Amount (mgm.)	Total activity (d.p.m.)	Specific activity (d.p.m./μmole)
1	Morphine	—	0·089 absorbed	621,000	2,000,000
		Morphine	1·53	337,000	62,800
		Codeine	0·088	none	—
		Thebaine	0·021	none	—
2	Codeine	—	0·12 absorbed	3,500,000	8,650,000
		Morphine	1·03	355,000	98,300
		Codeine	0·28	402,000	425,000
		Thebaine	0·042	none	—
3	Thebaine	—	0·04 absorbed	2,290,000	18,000,000
		Morphine	0·87	77,100	19,200
		Codeine	0·19	66,000	105,000
		Thebaine	0·026	29,900	321,000

Of the three alkaloids isolated from the morphine-fed plant, only morphine was radioactive; of those from the codeine-fed plant, only morphine and codeine were active; while from the thebaine-fed plant all three alkaloids were active. Since the alkaloids fed were from a biosynthesis with labelled carbon dioxide, they would be labelled both in the methyl groups and ring skeleton[4]. Hence it was necessary to demethylate and check ring skeleton activity in the isolated alkaloids in order to establish that incorporation of radioactivity was not simply due to transmethylation. The necessary degradations[1] were performed on thebaine which was fed to plant 3 and on the morphine and codeine isolated from that plant after 42 hr. It was found that the ratio of methyl group to ring skeleton activity was approximately the same in the thebaine as in the morphine and codeine isolated. Therefore, incorporation of radioactivity was not due to transmethylation, and the entire ring skeleton was converted to codeine and thence to morphine in accordance with the proposed scheme[1].

A comparison of the specific activities of the codeine and thebaine which were fed with the specific activities of the same alkaloids isolated at the end of 42 hr. shows that the final values are much lower than one would expect on the basis of pool dilution alone. This indicates a relatively rapid synthesis and conversion of the alkaloids, especially of thebaine, and a high percentage incorporation of specific activity would not be expected. Somewhat more meaningful



Morphine, $R = H$
Codeine, $R = CH_3$



Thebaine

in this case is the incorporation of total activity. Based on ring skeleton activities derived from the above-mentioned degradations, a 13 per cent incorporation was achieved from codeine to morphine in plant 2, while the incorporation from thebaine to morphine and codeine in plant 3 was 7·5 and 5·0 per cent, respectively. The values listed in Table 1 for the amount of alkaloid absorbed are based on loss of activity from the feeding solution. Experiments now under way show that a rapid adsorption or absorption of the alkaloids by the root hairs occurs, but that the further translocation is relatively slow. After the 42-hr. biosyntheses reported here, these root hairs had been removed and were not included in the total plant analysis. Therefore, the values for the percentage incorporation of total activity given above are undoubtedly minimal.

The proved conversion of thebaine to codeine and codeine to morphine clearly establishes O-demethylation as a metabolic pathway. This is in contrast to the commonly held concept[2] that the O-methylated alkaloids are metabolic end-products. Because of the relatively rapid synthesis and interconversion of alkaloids which has been found in this and the previous work[1], such demethylations could be a significant part of the total plant metabolism and perhaps provide one answer to the century-old problem of alkaloid function.

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Phenolase and Pectic Enzyme Activity in the Chocolate Spot Disease of Beans

The chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis fabae* Sard. is so named because an early result of infection by the fungus is a small brown lesion on leaf or stem. In the course of studying the factors controlling the development of these lesions, it was found that the latent phenolase of bean leaves could be activated by certain pectic substances and by a soluble cellulose derivative in a way similar to that described by Kenten[1] for anionic wetting agents. The result of phenolase action, with the naturally occurring leaf phenols as substrate, is an intense brown coloration.

Kenten[1,2] showed that aqueous extracts of bean leaves had very little phenolase activity, measured by oxygen uptake or by coloured end-products, unless extracts were first exposed to acid or alkaline conditions, precipitated by ammonium sulphate, or incubated with certain anionic wetting agents. In the present experiments, mixtures of dialysed leaf extracts, citrate/phosphate buffer and activators were incubated for appropriate periods. They were then added to catechol or dihydroxyphenylalanine (DOPA) at *p*H 7·0. Phenolase activity was measured in an EEL colorimeter with green filter 623. Kenten's results were confirmed with sodium dioctyl sulphosuccinate as the activating agent; the optimum *p*H for activation was 5·5, and at this *p*H the buffer alone had little effect. Solutions of sodium polypectate, polygalacturonic acid and pectin had an optimum *p*H for activation of 4·5, and the buffer alone had a slight activating action at this *p*H. Monogalacturonic acid also activated the phenolase, but was less effective than the polymers; with carboxymethylcellulose, activation was most rapid between *p*H 4·0 and 4·5.

It is likely that soluble pectic substances are liberated from walls during the invasion of tissues by *Botrytis fabae* because the fungus readily produces polygalacturonase in culture. Enzymatic hydrolysis of sodium polypectate and pectin to short-chain polymers did not destroy the ability to activate the phenolase.

Filtrates from cultures of *B. fabae* on certain media containing pectic substances rapidly reduce the viscosity of pectic substances in solution. These filtrates also disintegrate bean stem tissue, but if leaf extracts are included in the original media the filtrates have little or no polygalacturonase activity, and do not disintegrate stem tissue. Other results suggested that the enzyme was inactivated by substances formed following the oxidation of phenols in the leaf extract, and direct evidence for this was obtained in the following experiment. Phenolase was activated by carboxymethylcellulose at *p*H 4·0 and allowed to oxidize DOPA at *p*H 7·0 for 6 hr.; red-brown products had now developed. Equal volumes of oxidized DOPA and *B. fabae* culture filtrate were mixed at *p*H 7·0. Viscosity-reducing activity changed little over 2 hr. in the control with DOPA, but in the presence of oxidized DOPA, although there was no immediate effect, activity was markedly reduced after 10 min., and, after 1 hr., polygalacturonase activity was very low.

From these and other experiments, it would appear that the formation of a chocolate spot results from an interaction of the polygalacturonase systems of the fungus and the phenolase systems of the host. Fungal polygalacturonase probably kills host cells rapidly[3] and liberates the phenolase system. It also degrades cell walls and so liberates pectic substances which have been shown to activate host phenolase between *p*H 4·0 and 5·0. This will be followed by the oxidation of host phenols and the formation of dark brown polymeric products, which now inactivate the polygalacturonase of the pathogen.

A hypothesis based on interactions of this sort could explain why chocolate spot lesions are generally limited in size and why the tissue of the lesion remains coherent for some time.

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Absorption and Breakdown of Iron–Ethylenediamine Tetraacetic Acid by Tomato Plants

JACOBSON[1] was the first to show that plants could utilize iron supplied as the ferric chelate of ethylenediamine tetraacetic acid (Fe–EDTA). Subsequent workers[2,3] have concluded from experiments with isotopically labelled chelating agents that the iron was released after the uptake of the entire molecule by plant roots. Tiffin and Brown[4], however, showed that, at least initially, iron was absorbed by roots from a phenolic analogue of Fe–EDTA while most of the chelating agents remained in the culture solution. The absorption of Fe–EDTA was therefore further examined, using the iron chelate labelled in both portions of its molecule.

Tomato plants were raised from seed germinated in acid-washed sand and grown in an iron-free nutrient solution in the glasshouse. Ten days after the first true leaves had appeared, three plants of uniform size were transferred to each of six culture dishes. The nutrient solution included 1 p.p.m. of iron as Fe–EDTA, prepared from equimolar amounts of ferric chloride labelled with iron-59 and EDTA labelled with carbon-14 in the 2-position of the acetate group. The stability of the iron chelate in the nutrient solution was checked by keeping a further replicate free from plants under the same experimental conditions. During the experiment light was excluded from the nutrient solution and its *p*H was maintained at 5–6.

After 10, 17 and 24 days the plants and nutrient solution in two dishes were taken for isotope analysis. The plants in each dish were bulked, divided into tops and roots and dried at 65° C. overnight. For iron-59 assay, the material was digested with nitric and perchloric acids and the γ-radiation measured in a scintillation counter. For carbon-14, the plant material was oxidized with a Van Slyke acid mixture, the carbon-14 was collected as barium carbonate and counted under an end-window Geiger–Muller tube[5]. Solutions were evaporated to dryness in the presence of inactive carrier before oxidation and assay. No iron-59 was present in the barium carbonate precipitates. The iron-59 counts were converted to μgm. iron; carbon-14 results were expressed as μc. since the chemical form of this isotope was uncertain (Table 1).

Almost all the added iron was recovered either from the solutions or from the plants; by contrast the carbon-14 recovered fell steadily to about 60 per cent of its original value after 24 days, indicating decomposition of the EDTA. Both isotopes were almost completely recovered from the solution in which no plants were grown. The loss of carbon-14 was therefore not due to decomposition of the Fe–EDTA in the nutrient solution, but to absorption and breakdown in the plants.

After 10 days the plants had absorbed about 41 per cent of the added iron but only 26 per cent of the carbon-14. As there was no decomposition in the nutrient solution these figures imply that the plant roots had absorbed both iron and iron chelate. Later results showed that while the rate of uptake of iron decreased with time, that of the carbon-14 (EDTA) continued steadily. The EDTA liberated by the absorption of iron probably chelated other metals, for example, copper, zinc and manganese, in the nutrient solution. Subsequent absorption of these chelates would explain the steady increase in carbon-14 content and the progressive increase in the ratio of carbon-14 to iron in the plant tops.

This is the first report of experimental evidence that EDTA is broken down during the utilization by plants of the iron chelate, although it has been suggested previously that the iron might be released during breakdown of the chelate in leaves by sunlight[6].

Table 1

Duration of sampling date	Nutrient solution		Plant roots		Plant tops		Total recovery as percentage of original solution	
	μgm. Fe	μc. C-14	μgm. Fe	μc. C-14	μgm. Fe	μc. C-14	Fe	C-14
0 days (original solution)	200	9 04					100	100
10 days	122·7 111·5	6·98 6·29	14 6 15 0	0 30 0 40	55 6 65 0	0·11 0 11	96 5 95 8	81·8 75·2
17 days	75·0 90·0	5·47 5 92	22·9 31 3	0·82 0·59	93 4 68·9	0·24 0 17	95·7 95 1	72·2 73·9
24 days	81 0 84·6	3·87 4·49	30·0 29·4	0·81 0·57	81·1 70·6	0·35 0 63	96·1 92 3	55 6 62·9
24 days*	197 8	9·11	—	—	—	—	98·9	100·8

* This dish of nutrient culture solution contained no plants.

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A Lipoprotein Staining Method for Zone Electrophoresis

IN an attempt to overcome some of the well-known disadvantages of the commonly used lipoprotein stains, a new method, based on the use of Schiff's stain after preliminary ozonization, was developed. It does not depend on the use of lipid stains, and, consequently, washing the stain out of the background is not necessary. Furthermore, it can be used equally well on cellulose acetate material and on filter paper.

The electrophoretic separation is performed in the usual manner, but a somewhat larger sample than that used for ordinary protein separation is recommended. When the separation is completed, the strip is dried under a fan or in a hot-air oven at a temperature preferably not exceeding 80° C., and placed in a chamber for ozonization. The ozone can be introduced from an ozonizer or it can be generated chemically inside the chamber. I have been using a barium peroxide–concentrated sulphuric acid method which is both simple and cheap. A small glass container with the barium peroxide powder is placed inside the ozonizing chamber (a large jar or glass tank with a closely fitting lid, the size depending on the number and size of the strips) and a small volume of concentrated sulphuric acid is poured over the barium peroxide. The lid of the chamber is then quickly closed. The necessary amounts of the reagents will vary with the capacity of the chamber. After about 15–30 min. the strip is removed from the ozone atmosphere, rinsed in $N/1,000$ hydrochloric acid, and placed in Schiff's stain (prepared as for glucoprotein staining) until the lipoprotein bands acquire their maximum colour intensity. This usually takes about 5–15 min., but the staining time does not appear to be critical and the strips can be left for longer periods of time. When staining is completed, the strip is transferred into a reducing solution, for example, potassium metabisulphite crystals in $N/10$ hydrochloric acid. A thorough washing in three consecutive reducing baths is essential if the background is to remain colourless. A final wash in plain $N/10$ hydrochloric acid is recommended. The strip is then blotted and dried at room temperature. It was found that instead of the reducing bath, a 2–3 per cent aqueous nitric acid solution can be used with almost equally satisfactory results, with the advantage of not having to prepare the reducing solution each time and avoiding the irritating smell of sulphur dioxide. As an alternative to the preliminary exposure to ozone, quite satisfactory results can also be obtained by immersing the strip for about 20 min. in a 2–3 per cent solution of iodic acid, or in a 5 per cent aqueous potassium iodide solution which has been left standing in the light for a few days acquiring a very pale yellow tinge. It should then be kept in the dark. The intensity of colour of the lipoprotein bands, however, will not reach that obtained after ozonization.

The results are shown in Figs. 1 and 2. In normal human serum two lipoprotein fractions are usually seen, but it should be noted that on cellulose acetate the main lipoprotein fraction of the serum travels with α_2 mobility and not with the β fraction as on filter paper. The stained bands obtained by this method coincide exactly with those obtained with any of the conventional lipid stains such as sudan black. This can be seen by comparing the bands with parallel lengths from the same strip by the ozone – Schiff and sudan black methods respectively (Fig. 1).

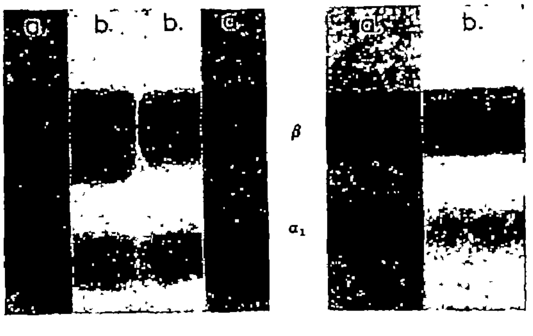

Fig. 1. Comparison of electrophoretic lipoprotein patterns as obtained by: (a) sudan black stain; (b) ozone–Schiff method. Electrophoretic run on same strip of filter paper (courtesy of Dr. W. G. Dangerfield)

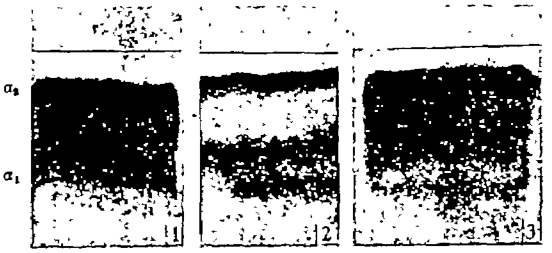

Fig. 2. Lipoprotein patterns of human serum on cellulose acetate obtained by (1) ozone–Schiff method; (2, 3) potassium iodide–Schiff method

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PHYSIOLOGY

Some Pharmacological Actions of D-Lysergic Acid Methyl Carbinolamide

D-LYSERGIC acid methyl carbinolamide, $C_{18}H_{21}N_3O_2$, is a new alkaloid produced by a strain of *Claviceps paspali* Stevens and Hall. Arcamone, Bonino, Chain, Ferretti, Pennella, Tonolo and Vero[1] isolated the new alkaloid from submerged cultures and determined its structure. I have now investigated some of the pharmacological actions of the new alkaloid.

D-Lysergic acid methyl carbinolamide was kindly supplied to me by Prof. E. B. Chain. Freshly prepared aqueous solutions of the maleate salt ($C_{18}H_{21}N_3O_2\ C_4H_4O_4$) were used in my experiments.

The intravenous *LD*50 of the new alkaloid was approximately 150 mgm./kgm. for mice and 0·75 mgm./kgm. for rabbits. Before death, the mice showed periodic convulsions of a clonic type, erection of the hairs and excitability. At doses of 50–100 mgm./kgm. it produced only this peculiar symptomatology: the mice stood upright and pressed on each other's noses and chattered their teeth. In rabbits, an injection into the ear vein in doses of 0·1–1 mgm./kgm. produced dilatation of the pupil, excitability or convulsions of a clonic type. The ears became pale and cold with intense vaso-constriction. The toxicity of the alkaloid to rabbits seemed to depend on its power of raising the body-temperature in this species. In rabbits the similarity between the effects of the new alkaloid and ergometrine were particularly striking, but ergometrine was less toxic to rabbits (the approximate intravenous *LD*50 of ergometrine was 3·5 mgm./kgm.).

D-Lysergic acid methyl carbinolamide induced, in low concentrations (minimum active concentration 0·1–1 μgm./ml.), a contracture in the isolated uterus of the virgin guinea pig. There was a satisfactory dose/response relationship. This contracture was very similar to that produced by ergometrine maleate, which, however, was 1–2 times more potent.

On the rabbit uterus *in situ* both alkaloids produced a prompt contraction and increased rhythmic activity of the uterus. For ergometrine the minimum active dose by intravenous route was 0·1–0·3 mgm./kgm. and for the new alkaloid 0·2–0·5 mgm./kgm. The actions of both alkaloids lasted some minutes, and owing to the favourable circumstance that the interference between the effects of the two alkaloids was negligible, it was possible to test them on the same preparation. Ergometrine was 1–2 times more potent than the new alkaloid.

On the isolated seminal vesicles of the guinea pig, the new alkaloid was approximately 200 times less potent than ergotamine tartrate as an adrenergic blocking drug.

Rabbits anæsthetized with urethane supported doses of D-lysergic acid methyl carbinolamide which would have killed unanæsthetized animals. Rapid intravenous injections of small doses (0·1–0·2 mgm./kgm.) of the new alkaloid caused an evanescent decrease or a small increase of blood pressure; with higher doses (0·3–0·5 mgm./kgm. and more) the blood pressure increased moderately without showing any dose/response relationship. Ergometrine maleate seemed to be less active on blood pressure, and there was no significant change of blood pressure with 0·3–0·5 mgm./kgm.

The new alkaloid was without effect, when given in small doses, on the blood pressure of cats anæsthetized with chloralose. Higher intravenous doses (0·1–0·3 mgm./kgm.) caused a sustained hypotension of long duration and a moderate decrease of heart-rate. The respiration of rabbits and cats was depressed by small doses of the new alkaloid; cats seemed to be less resistant than rabbits. In cats, 0·01 mgm./kgm. of the new alkaloid caused broncho-constriction and contraction of the nictitating membrane of long duration.

The new alkaloid had no action on isolated rabbit auricles at doses up to 100 μgm./ml.

In summing up, the new naturally occurring alkaloid D-lysergic acid methyl carbinolamide has powerful ergometrine-like oxytocic actions and weak ergotamine-like adrenergic blocking actions. It must be included, on the basis of pharmacological evidence, in the ergometrine group of ergot alkaloids. Ergometrine, however, is less toxic and more active than the new alkaloid. Some of my results suggest that it could have a lysergic acid diethylamide-like activity, but this hypothesis must be checked by experiments on humans.

Full details of this work will be published elsewhere.

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Repression and De-repression of Enzyme Synthesis as a Possible Explanation of some Aspects of Drug Action

ACCORDING to recent theories, the induction of enzyme synthesis occurs as a result of the displacement from an enzyme-forming system of a natural repressor[1]. For example, the synthesis of alkaline phosphatase by *E. coli* is normally repressed by inorganic phosphate[2]. De-repression occurs when there is only a small amount of inorganic phosphate in the medium. The repressor theory is in good agreement with most of the observations which have been made on enzyme-inducing systems. The inducer need not be a substrate for the enzyme which is formed, nor need it serve as a template for the formation of new enzymes[1].

While this phenomenon is best known from work with bacteria, examples of the de-repression of various mammalian enzymes have also been reported[3]. Especially interesting are instances of the apparent de-repression of enzymes which metabolize certain drugs and carcinogens. There is an enzyme system in liver microsomes which, in the presence of reduced triphosphopyridine nucleotide and oxygen, can carry out a number of 'detoxifying' reactions. These include: the hydroxylation of aromatic rings, the O-dealkylation of aromatic ethers, the N-dealkylation of substituted amines, reductive cleavage of diazo groups, and the oxidation of alkyl side-chains. There are probably several different enzymes involved, but they all have similar requirements, and all are inhibited by diethylaminoethyl-diphenylpropyl acetate (*SKF 525-A*)[4].

Many of the known substrates of the reduced triphosphopyridine nucleotide – oxygen – microsomal system, and some compounds of similar structure, can produce marked de-repression of various enzymatic activities when injected into young rats. Examples of such increased enzyme synthesis are: increased hydroxylation of 3,4-benzpyrene[5] and 2-acetylaminofluorene[6]; and increased N-demethylation and reductive cleavage of azo dyes[7,8]. Actual enzyme synthesis is believed to be taking place in these instances because treatment with ethionine prevents such increases in enzyme activity and this inhibition is overcome by methionine.

Another enzyme system which can be de-repressed by the same treatment is that which converts glucose to glucuronic acid and ascorbic acid. This de-repression leads to striking increases in excretion of ascorbic acid and glucuronic acid by treated rats [8–10]. There is also increased conversion of glucose-^{14}C to glucuronic acid and ascorbic acid[9]. The increased excretion of ascorbic acid appears considerably later than the rise in activity of the reduced triphosphopyridine nucleotide–oxygen–microsomal system, and may perhaps be a secondary response to increased synthesis of glucuronic acid. It can be pointed out that the detoxified products of the microsomal system are usually further metabolized as glucuronides[4]. While barbital is not metabolized by the microsomal system which 'detoxifies' other barbiturates, it can nevertheless produce the same type of de-repression[8].

It may well turn out that the physiological substrates of both the 'detoxifying' microsomal system and the glucuronide-synthesizing system are actually steroids. The injection of 3-methyl cholanthrene or related carcinogens increases the hepatic metabolism of hydrocortisone[11]. Compounds such as methyl cholanthrene, benzpyrene and dibenzanthracene could act as de-repressors by virtue of their similarity to natural steroid repressors. These carcinogens are known to potentiate the biological activities of steroids[11]. There is a sex difference in the activity of the microsomal system in adult rats. This produces marked differences in the rates of detoxification of hexobarbital[4] and the N-demethylation of morphine[12]. Such sex differences can be altered in either direction by treatment with sex hormones, and this may represent an example of repression and de-repression by steroid hormones.

Examples of drug-induced enzyme repression can also be found. An outstanding case is the striking decrease in N-demethylase activity found by Axelrod in the liver microsomes of rats made tolerant to morphine[13]. This was suggested as a model system for the phenomenon of drug tolerance as seen in narcotic addiction[13,14]. It was postulated by Axelrod that continued exposure to morphine might lead to a similar inactivation of a receptor site in the central nervous system. This theory does not explain the development of physical dependence as manifested by the appearance of withdrawal symptoms.

The concept of drug-induced repression of enzyme synthesis can be used to formulate a scheme which serves to explain the features of tolerance to, and physical dependence on, narcotics like morphine as shown in Fig. 1.

According to this scheme, morphine can act at two sites. By inhibiting at site *A* the action or synthesis of a neurohormone required for proper functioning of the central nervous system, it can bring about its characteristic effects of depression of the central nervous system, analgesia and sedation.

On repeated administration (or on a few treatments with very high doses[14]) morphine also begins to act at site *B*, causing de-repression of the system which makes the neurohormone-synthesizing enzyme. This would increase the amount of neurohormone acting on the central nervous system. Tolerance to the depressant effects of morphine develops because more morphine is now required to depress the central nervous system.

Another way in which the same result could be achieved is also indicated in Fig. 1. Instead of acting at site *B*, morphine could increase the concentration of neurohormone by repressing, at site *C*, the synthesis of an enzyme which destroys the neurohormone. Such a repression would be similar to the repression by morphine of the synthesis of morphine N-demethylase.

When morphine is afterwards withdrawn, the increased amount of neurohormone is no longer 'neutralized' by morphine, and the result is the excitation of the central nervous system typically observed as withdrawal symptoms. This creates an apparent physical dependence on the narcotic, since these symptoms are readily controlled by a resumption of morphine administration. The withdrawal symptoms gradually diminish in intensity as the concentration of neurohormone returns to normal and tolerance also disappears. The morphine antagonist N-allylnormorphine can precipitate withdrawal symptoms in an addicted animal by competing with morphine for site *A* and thus unmasking the effects of an increased concentration of neurohormone.

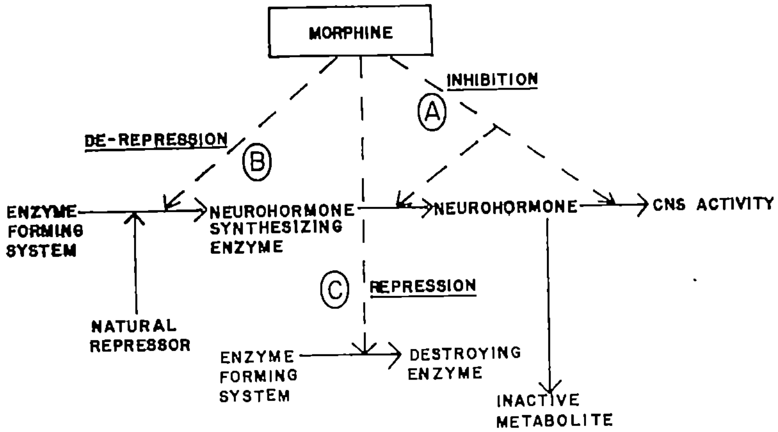

Fig. 1. Hypothetical scheme for explaining some of the actions of morphine in terms of repression and de-repression of enzyme synthesis

The examples given here indicate that the concept of repression and de-repression of enzyme synthesis may prove useful in understanding how drugs can modify the biochemical mechanisms which make for a normal homœostatic condition in the mammalian organism.

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Glutamic Oxaloacetic Transaminase Activity in Different Zones of the Ventricular Muscle of the Dog

OF the tissues of several animal species the myocardium has the highest concentration of glutamic oxaloacetic transaminase (GOT)[1-3]. Distribution of this enzyme in several zones of the cardiac muscle has received but scant attention in the literature. However, it has been shown that the dog's left ventricle has a higher GOT activity than the right one[4], and a similar difference has been described for the concentration of adenosine triphosphatase and succinic dehydrogenase[5]. On the other hand, Jennings, Kaltenbach and Smetters failed to note significant differences in the left ventricle of the dog between the GOT activity of the posterior papillary muscle, the left ventricle immediately beneath, and the anterior superior septum[6]. The present observations show that GOT activity of the myocardium is not uniform, and that there are significant differences in dogs among several zones of both ventricles.

Six male and four female adult mongrel dogs, weighing between 5 and 8 kgm. were anæsthetized with sodium pentobarbital (33 mgm./kgm.); the heart was removed and two small muscle samples from each one of the following zones of the ventricles were excised: (*a*) outer surface from the middle part of the free wall (to be called left epicardium); (*b*) inner surface of the free wall beneath the removed epicardial zone (to be called left endocardium); (*c*) muscle lying between the two formerly described (to be called mesocardium); (*d*) inner endocardial layers from the middle part of the left side of the septum (to be called septum). In six further dogs (four male and two female), (*a*) and (*b*) were removed simultaneously with (*e*) outer surface from the middle part of the right free wall (to be named the right epicardium) and (*f*) inner ventricular surface beneath the previous one (to be named right endocardium). Twenty mgm. were immediately weighed from each sample in a torsion balance to the nearest mgm., and cut in 10-µ sections with a freezing microtome. The enzyme was extracted by incubation at 2–4° C. for 30 min. in phosphate buffer at *p*H 7·4. Enzyme activity was determined by a modification of the technique of Cabaud, Leeper and Wroblewski for blood serum[7]. Incubation was carried out at 25° C. for 20 min. with D,L-aspartic and α-keto glutaric acids as substrate. All observations were performed 'blindly' in duplicate; results were compared with Student's t test according to Fisher and Yates's Tables[8].

The results expressed in µmoles of pyruvate liberated per mgm. of wet tissue under the stated conditions are shown in Tables 1 and 2. Error of the method ($e = \frac{Sm \times 100}{\bar{X}}$; where Sm = standard error of the mean; $\bar{X}$ = mean of observations) was found to be ± 0·81 per cent on 16 duplicate determinations and performed on one ventricular sample. As can be seen the GOT activity of the left endocardium (1·126 ± 0·041 *S.E.*) is higher than that of the left epicardium (0·817 ± 0·036). The enzymatic activity of the mesocardium (0·946 ± 0·029) lies between both, while in the septum (0·971 ± 0·027) it is lower than in the left endocardium. No significant differences in enzymatic activity are noted between the septum and the mesocardium. On the other hand, the right epicardium has a higher concentration than the right endocardium (1·205 ± 0·088 and 0·949 ± 0·063, respectively).

The high concentration of the GOT in the myocardium[1–3] may indicate that this enzyme has an important bearing on cardiac metabolism. Nevertheless, very little is known about the relationships existing between such an enzymatic activity and the main manifestations of cardiac activity, namely, bioelectric and contractile phenomena. Transamination reactions provide anaerobically certain metabolites (oxaloacetic and pyruvic acids, for example) which are aerobically oxidized stepwise by mitochondrial enzymes of the Krebs cycle, liberating a great amount of energy. In this connexion it has been pointed out that the cardiac muscle can use these metabolites to supply a large part of the energy required to support the contractile activity of the myocardium[9].

Table 1. GOT ACTIVITY IN DIFFERENT ZONES OF THE LEFT VENTRICLE

Zone	No. of determinations	GOT in µmoles of pyruvate/ mgm. of wet tissue
(1) Epicardium	20	0·817 ± 0·036
(2) Endocardium	20	1·126 ± 0·041
(3) Mesocardium	16	0·946 ± 0·029
(4) Septum	16	0·971 ± 0·027

t 1 vs. 2 = 5·722, $P < 0·001$; t 1 vs. 3 = 2·866, $P < 0·01$; t 1 vs. 4 = 3·500, $P < 0·01$; t 2 vs. 3 = 3·660, $P < 0·001$; t 2 vs. 4 = 3·875, $P < 0·001$; t 3 vs. 4 = 0·833.

Table 2. GOT ACTIVITY IN DIFFERENT ZONES OF BOTH VENTRICLES

Zone	No. of determinations	GOT in µmoles of pyruvate/ mgm. of wet tissue (mean ± standard error of the mean). Right ventricle	No. of determinations	GOT in µmoles of pyruvate/ mgm. of wet tissue (mean ± standard error of the mean). Left ventricle
(1) Epicardium	11	1·205 ± 0·088	11	0·956 ± 0·061
(2) Endocardium	12	0·949 ± 0·063	10	1·201 ± 0·077
t 1 vs. 2		2·370, $P < 0·05$		2·525, $P < 0·01$

It is probable that differences in enzymatic activity may be related to functional differences of the myocardium, since metabolic activity determines the fundamental properties of the cardiac fibres. Our findings indicate that the free wall of the left ventricle shows an enzymatic concentration that falls progressively from the endocardium to the epicardium. Furthermore, the samples from the left endocardial surface of the septum show a lower enzymatic activity than those from the endocardium of the left ventricular free wall. On the other hand, in the right ventricle the epicardium has a higher enzymatic concentration than the endocardium. When it is considered that some of the bioelectric properties, for example, rate of repolarization, are not the same in different zones of the myocardium[10], it seems worthwhile to study the relationships, if any, of these properties and transamination activity.

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Inhibiting Effect of 2 : 4-Dinitrophenol on Calciferol-induced Metastatic Calcification in the Rat

PREVIOUS investigations by Trout[1] and by us[2] indicate clearly that the deposition of mineral in the aortæ of calciferol-intoxicated rats continued long after cessation of administration of this compound and after the blood calcium-level had returned to normal. It appears that, once initiated, this process follows an irreversible course, at least in the aorta, since even 160 days after the initial insult there was no evidence of a reduction in the mineral content of the aorta. Attempts to inhibit calciferol-induced calcification of the aorta by the simultaneous administration of papain (which reduces the mucopolysaccharide content of the aorta) were unsuccessful[3].

Since the calcification is accompanied by a large increase in aortic phosphorus content, it was decided to investigate the effect of 2 : 4-dinitrophenol (DNP) on the action of calciferol administered simultaneously; for it has been shown by Whitehead and Weidman[4] that DNP *in vivo* inhibits the synthesis of adenosine triphosphate, as measured by the incorporation of phosphorus-32, and also the incorporation of the isotope into the bone salt of kitten cartilage. Gutman and Yü[5] found that calcification *in vitro* was markedly inhibited by 2 : 4-dinitrophenol at 10^{-4} *M* concentration. Whitehead and Weidman suggested that the relationship between phosphorus-32 incorporated into bone salt and adenosine triphosphate is that the latter is required to initiate the formation of a primary seed which acts as a nucleus for the subsequent deposition of bone salt.

Accordingly, 20 male Wistar strain rats, initially weighing approximately 250 gm., were divided randomly into four groups of five. Group I was given, twice daily, intraperitoneal injections of 3 ml. of 0·9 per cent sodium chloride solution for twenty days. Group II received identical saline injections, together with calciferol (a total of 100,000 units/kgm. body-weight) given orally, in daily doses, as a solution in arachis oil from day 6 to 10 inclusive. Group III were given, twice daily, injections of 3 ml. of a saturated solution of DNP in 0·9 per cent sodium chloride, while Group IV received the same injections together with calciferol orally as in Group II. On day 20 the animals were killed and the aorta, lungs and stomach dissected out. The tissues were dehydrated with acetone, defatted with ether–alcohol (1 : 1 v/v) and dried to constant weight at 100° C. A second series of animals received the same treatment, but were killed on day 9 of the experiment, that is, 4 days after starting calciferol treatment, and bled from the heart; the serum was analysed for calcium and inorganic phosphorus. Differences between means were assessed by the Mann–Whitney *U* test, using the 5 per cent level of significance.

Table 1 shows the mean aortic dry weight and the calcium and phosphorus contents of the aortæ. Calciferol treatment resulted in an approximately two-and-a-half-fold increase in mean aortic dry weight, similar to that previously found by us[2]. We have found this increase in weight to be due largely to the increased mineral content of the aortæ (see below). For this reason, the results for calcium and phosphorus are expressed in mgm./aorta. It will also be seen from Table 1 that DNP, administered simultaneously with calciferol, significantly reduced the effect of calciferol on total aortic weight, although DNP alone had no effect at all on the aortic dry weight.

Table 1. DRY WEIGHT AND MINERAL CONTENT OF RAT AORTÆ

Group	Treatment	Dry fat-free weight (mgm.)	Calcium (mgm./aorta)	Phosphorus (mgm./aorta)	Calcium/phosphorus ratio
I	Saline only	16·7 ± 1·2*	0·21 ± 0·06	0·07 ± 0·01	3·0 : 1
II	Saline + calciferol	48·8 ± 5·5	6·23 ± 0·91	2·30 ± 0·33	2·7 : 1
III	DNP only	16·7 ± 1·0	0·11 ± 0·01	0·08 ± 0·01	1·4 : 1
IV	DNP + calciferol	28·1 ± 4·0	2·88 ± 1·04	1·15 ± 0·31	2·5 : 1

* Mean ± *S.E.*

Calciferol treatment also resulted in significant increases in both calcium and phosphorus contents of the aorta; DNP alone had no effect on these constituents, but when given simultaneously with calciferol, significantly reduced the degree of mineralization induced by calciferol, by approximately one-half. The calcium–phosphorus ratio of the aortæ tended to be reduced in the two groups receiving DNP injections, but, due to wide variations in individual values, the differences were not significant.

Results similar to those from aortæ were obtained for lung and stomach; for calciferol produced significant increases in the calcium and phosphorus contents of these organs as well[2] and DNP, given simultaneously, reduced this effect.

The results of the serum calcium and phosphate determinations showed that calciferol markedly increased both these serum constituents (calcium–phosphorus product raised to 125 compared with control value of 77). However, despite its marked effects on tissue mineralization, DNP given simultaneously with calciferol did not affect the increases in serum calcium and phosphorus induced by calciferol.

Hence it would appear that the inhibiting effect of DNP on the calcification induced by calciferol is not mediated via any overt action on blood calcium or phosphate-levels. It would thus seem to exert some direct effect on the tissues, although an indirect action, via the endocrine system, for example, parathyroids, cannot be ruled out at present.

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Function of the Hypophysis–Thyroid System in the Rabbit Fœtus with Regard to Cholesterol Metabolism

THE experimental studies of Jost[1] and Hwang and Wells[2] have demonstrated the functional interrelationship of the pituitary and thyroid in the rabbit and rat fœtus. Rumph and Smith[3] showed that the thyroid of the 90-mm. pig fœtus contains a substance which would induce metamorphosis in the hypophysectomized tadpole. Geloso[4] demonstrated the presence of thyroid hormones in the blood of the rat fœtus. Jost and Picon[5] showed that fœtal thyroidectomy resulted in an increase in total body fat in the rabbit fœtus. Bearn[6] found an increase in liver fat following hypophysectomy by decapitation in the rabbit fœtus, and correlated this with the increase in the fat content noted in human anencephalic fœtuses. These experiments can be taken to indicate that the fœtal thyroid is capable of function, and that its function is regulated by the fœtal pituitary.

It is well known that there is in both adults and in infants a close relationship between thyroid function and cholesterol metabolism. A rise in blood cholesterol has been reported in hypothyroidism[7] and in cretinism[8]. It was therefore decided to investigate the role of the pituitary and thyroid of the rabbit fœtus in the regulation of cholesterol metabolism.

Nineteen rabbit fœtuses were decapitated by a modification of the method described by Jost[9]. Nine fœtuses were decapitated through the neck to remove the thyroid as well as the pituitary, and ten were decapitated through the mouth to preserve the thyroid. The operation was performed between days 21 and 23 following witnessed mating, and the fœtuses were recovered after seven days of further intrauterine development; 53 litter mates were used as controls.

The cholesterol-level of the blood was determined blind by the method described by Abell *et al.*[10], and the results expressed in mgm. per 100 ml. As used in this laboratory, the method has a standard error of blind duplicates of 8·3 mgm. The results are summarized in Table 1.

Table 1. CHOLESTEROL-LEVELS IN SERUM OF DECAPITATED AND CONTROL FŒTUSES

Fœtuses	Age at investigation	No.	Mean serum cholesterol (mgm./100 ml. ± S.E.)
Low decapitation (at 21–22 days)	28–29 days	9	142 ± 5·2*
Litter mate controls	28–29 days	29	87 ± 2·9*
High decapitation (at 22–23 days)	29–30 days	10	156 ± 6·5*
Litter mate controls	29–30 days	24	97 ± 4·2*

* These differences are significant, $P < 0.001$.

There is a variability in the level of blood cholesterol from one litter to another, but the level in each of the control fœtuses from a single litter shows little variation.

The decapitated fœtuses all showed a significant rise in blood cholesterol, even in those fœtuses in which the thyroid was left behind and only the pituitary removed.

These preliminary results indicate that the fœtal pituitary and thyroid play a part in the cholesterol metabolism of the fœtus. In addition, they indicate that maternal thyroid hormones and maternal thyrotropic hormone do not compensate for the absence of fœtal thyroid and pituitary hormones in the rabbit.

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Rate of Anaerobic Glycolysis versus Structure in Pork Muscle

THE *p*H of pork musculature after cessation of post-mortem glycolysis (ultimate *p*H) has long been known to be decisive for structure and water-holding capacity of the muscle. Recent observations[1,2] have shown that considerable variation in structure of muscle with the same ultimate *p*H may be recorded. The rate of the post mortem *p*H fall was found to be of considerable importance.

The investigations which are briefly reported here were facilitated with a *p*H meter (Radiometer model No. 31; electrode-elector type *ELS*31; electronic recorder type 153, Honeywell) for continuous and simultaneous *p*H and temperature recordings. Six *p*H electrode pairs (glass electrodes *G* 2326, calomel electrodes 4116) were inserted in representative muscles and permitted to remain in position from 1 to 24 hr. post mortem. Careful tests have not revealed any indications of artefacts arising from this procedure. The carcasses from 20 Danish Landrace pigs (85–95 kgm.) were used in this work. The continuous recordings from these carcasses depict at least four distinct types of post mortem *p*H patterns: (1) A slow gradual decrease to an ultimate *p*H of 5·7–6·3. (2) A gradual decrease to approximately 5·7 at 8 hr. with an ultimate *p*H of 5·3–5·7. (3) A relatively rapid decrease to approximately 5·5 at 3 hr. with an ultimate *p*H of 5·3–5·6. (4) A sharp, significant decrease to a *p*H of approximately 5·1 at 1½ hr. and a subsequent elevation to 5·3–5·6.

The anaerobic glycolysis of the type 4 compared to the type 2 *p*H pattern and the effect on the tissue of the longissimus dorsi muscle will be briefly elaborated.

A Radiometer *p*H meter (model 24) was used to determine *p*H values at 15-min. intervals to the 1-hr. period while the carcasses were in the slaughter line. Afterwards the carcasses were transferred to a 3° C. atmosphere for continuous recordings (*p*H

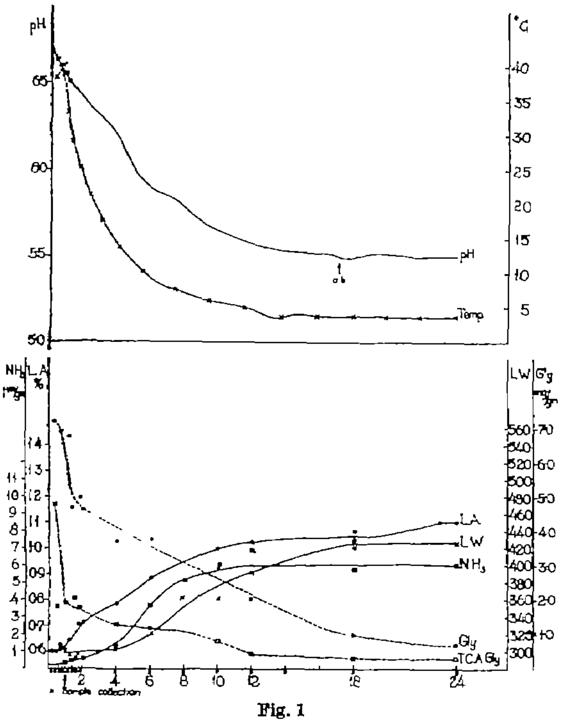

Fig. 1

and temperature) from 1 to 24 hr. Thermocouples and electrodes were uniformly placed in the muscle in the area of the second lumbar vertebra. Samples of the longissimus dorsi from the twelfth rib to the last lumbar vertebra of the opposite side were secured, for analysis, at various intervals as indicated in Fig. 1.

The results in Fig. 1 show the features of a slow rate of anaerobic glycolysis (type 2), those in Fig. 2 depict the rapid rate of the type 4 muscle. In the type 4 muscle *p*H decreased to 5·1–5·2 in 1½ hr., while the temperature was 36–40° C. In the type 2 muscle the lowest *p*H around 5·5 was not reached until the temperature approximated 4° C. The difference in the rate of *p*H fall was due to difference in rate of anaerobic glycolysis as illustrated by the glycogen[1] and lactic acid[3] determinations. The type 4 muscle initially contained a relatively high percentage of protein-bound glycogen. This may be seen by comparison of the amounts of glycogen soluble in trichloracetic acid in a single rapid extraction[4,5]. The liberation of ammonia[6] and depletion of labile phosphate (7'P) (ref. 7) suggest that rigor mortis occurred during the first 30–45 min. in the type 4 muscle, compared to 4–6 hr. post mortem for the type 2 muscle. It was furthermore noticed that the decrease in temperature during the first 2 hr. was comparatively slow in the type 4 muscle, apparently due to the heat of the rapid glycolytic reactions.

The effect of *p*H and onset of rigor mortis on the water-holding capacity of the tissue is depicted by the 'loose-water' (LW) number which gives the amount of water in mgm. which can be pressed out of 1 gm. meat under standardized conditions[1]. In the type 4 muscle these numbers rise very sharply to high values and the meat assumed a pale colour. These changes *suggest partial denaturation of the meat proteins*[1].

The sharp pronounced elevation in the type 4 *p*H curve appears connected to partial denaturation of the muscle proteins. In this context it is interesting to note that the proteins of the type 4 muscle show increased affinity for safranine and orange *G* dyes which have been used to determine acidic and basic groups[8]. Furthermore, these proteins bind increased amounts of congo red dye, which is regarded as a sign of denaturation[9]. Average results are reported in Table 1.

Table 1. DYE-BINDING PROPERTIES (24-HR. SAMPLES)

	Type 2	Type 4
Orange *G**	13·94	17·70
Safranin*	14·44	18·71
Congo red†	8·40 (7·79)‡	9·13 (7·31)‡

* Equiv. per 10^4 gm. protein.
† Mgm. dye-bound/100 mgm. protein.
‡ 15 min. post mortem.

Further support for the importance of the acid–temperature relationship has been shown by repeated trials with sections of the longissimus dorsi muscle. These sections were removed from the carcasses, immediately post mortem, and held at varied temperatures. The sections which were retained at 37° C. for 1½ hr. displayed the characteristics of type 4. Conversely, those which were chilled rapidly seemingly avoided the extreme drop in *p*H and were normal in colour and structure.

Preliminary observations of biopsy samples from six Danish Landrace pigs which ultimately showed the type 2 and type 4 *p*H curves are shown in Table 2. Three weeks prior to slaughter the pigs were anæsthetized with trichloro-ethylene and biopsy samples were removed from the lumbar region of the longissimus dorsi muscle[10]. The biopsy samples of pigs which ultimately showed the type 4 *p*H pattern contained greater total glycogen, higher percentage of protein-bound glycogen and faster phosphorylase activity. Similarly, these pigs possessed a smaller pyruvic acid pool and a greater lactic acid

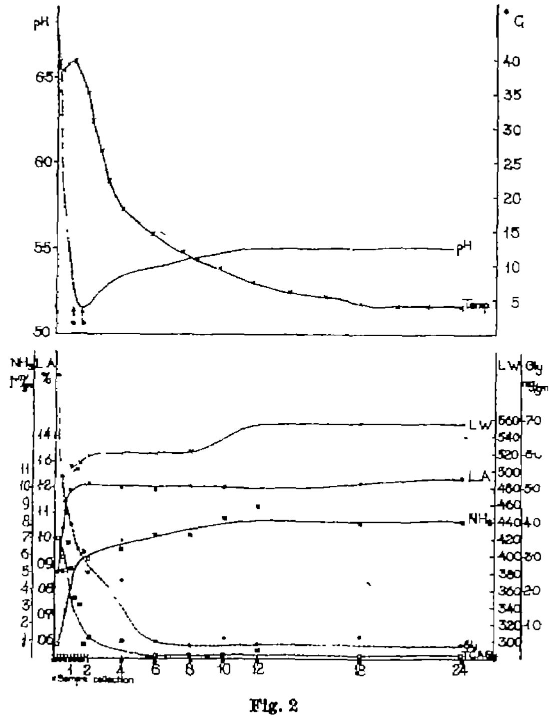

Fig. 2

Table 2. GLYCOLYTIC COMPONENTS (BIOPSY SAMPLES)

	Type 2	Type 4
Total glycogen (mgm./gm.)	4·02	7·45
Glycogen (acid-soluble) (per cent of total)	49 64	39 39
Pyruvic acid (mgm. per cent)	2 80	1·18
Lactic acid (per cent)	0·29	0 52
Phosphorylase (mgm. glycogen/gm. tissue/30 min.)	11·38	15 49

concentration. Results to date also suggest that lactic acid is converted to pyruvic acid, *in vivo*[11], at a slower rate in muscles with the type 4 *p*H pattern.

It is hoped to publish a complete account of these investigations elsewhere. One of us (E. J. B.) is grateful to Oscar Mayer and Co., Madison, Wisconsin, for their support (on leave from University of Wisconsin) for the duration of this work.

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HÆMATOLOGY

Deformation of Red Cell Shape induced by *Vipera palestinae* Venom *in vivo*

IT has been previously reported[1] that *Vipera palestinae* venom causes *in vitro* disk-sphere transformation of red blood cells in the presence of plasma without producing hæmolysis. This phenomenon could be reversed by suspending the spherical red cells in normal plasma. In the present work an attempt was made to establish whether similar changes of red blood cells occur also *in vivo*.

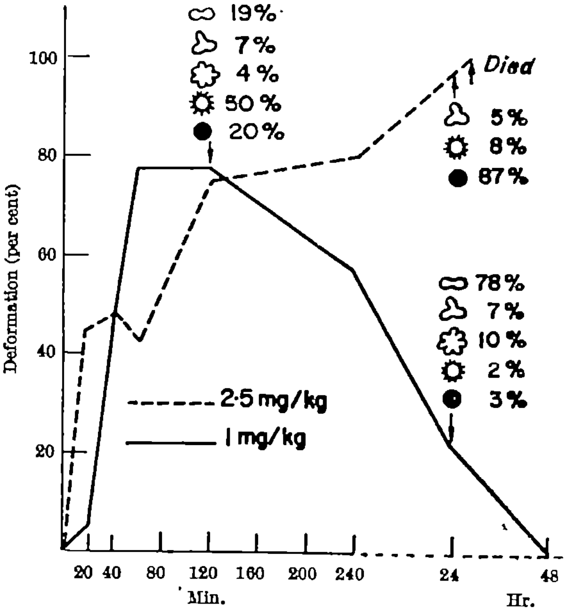

Fig. 2. *In vivo* deformations of erythrocytes of unanæsthetized rats after intramuscular injection of *Vipera palestinae* venom

Sublethal and lethal doses of venom were injected by the intramuscular and intravenous routes to rabbits and rats anæsthetized with urethane or pentobarbital sodium as well as to non-anæsthetized animals. A drop of rat blood from the tail tip was introduced directly into 0·5 ml. of 0·5 per cent osmium tetroxide solution in phosphate buffered isotonic sodium chloride, *p*H 7·2. Rabbit blood samples were drawn from the ear vein or from the jugular vein. Blood cells were examined under the phase contrast microscope, or, after a single washing of the fixed cells with distilled water, in aluminium shadow-cast smears[2], in which the various shapes of 200 erythrocytes were counted. The degrees of deformation observed were classified as follows: (1) normal bidiscoids; (2) slightly deformed cells (the bidiscoid form no longer recognizable, but not yet crenated); (3) grossly crenated cells; (4) finely crenated spheres of small diameter; (5) spheres of small diameter.

In unanæsthetized non-venom-treated animals 95–100 per cent of the red cells were bidiscoid. The majority of the anæsthetized non-venom-treated animals, however, showed some degree of deformation of the red blood cells, ranging from practically 0 to 37 per cent slightly deformed erythrocytes (degree 2). After intramuscular injection of venom the degree of deformation as well as the percentage of cells with a higher degree of deformation increased with time. With increasing doses the changes occurred more rapidly.

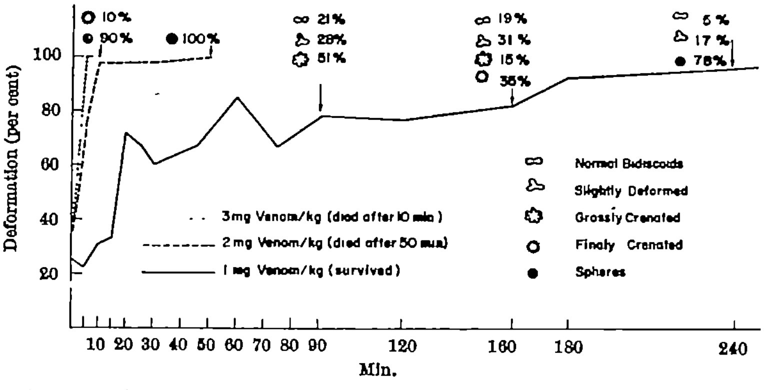

Fig. 1. *In vivo* deformations of erythrocytes of anæsthetized rabbits after intramuscular injection of *Vipera palestinae* venom

Fig. 1 presents the progressive deformation of erythrocytes of anæsthetized rabbits injected intramuscularly with sub-lethal and lethal doses of venom. The curves represent the percentage of deformed cells, counted after various time-intervals.

Sublethal doses (up to 1 mgm./kgm. intramuscular) induced maximum deformation after about 3 hr. Thereafter reversion to the normal bidiscoid shape began, reaching completion after 6–48 hr. Lethal doses produced rapid progressive deformations, culminating in complete sphering; the higher the venom dose, the earlier this occurred.

Fig. 2 represents the findings in unanæsthetized rats. When a lethal dose (2·5 mgm./kgm. intramuscular) was injected the deformation reached a maximum shortly before the animal died, while no ghosts were observed. This is in agreement with the venom having no direct hæmolytic activity *in vitro*[3]. On the other hand, injection of the venom of *Naja naja* (cobra), known to have direct hæmolytic activity, did produce ghosts. After administration of a sublethal dose (1 mgm./kgm.) reversion of the deformation occurred and was completed after 24–48 hr.

In view of these results it is suggested that the severity of *Vipera palestinae* snake bite might be judged by the study of the degree of deformation of the red cells in a sample of peripheral blood, fixed in osmium tetroxide.

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Di^a, Js^a and V Blood Groups in South and South-East Asia

The red-cell antigens, Di^a and Js^a, are among those of special interest as genetic markers in human populations because they attain reasonable frequency in at least one of the major divisions of the human race, but are rare or entirely absent in all others. The Di^a blood-group factor is restricted to American Indians and Mongoloid populations[1] or those of mixed American Indian ancestry[2]. The more recently discovered Js^a antigen has been detected so far only in the blood of Negroes or of those with the possibility of Negro ancestry[3]. A third blood-group, the Rh antigen V, also may be highly restricted, since it is found most commonly in the blood of Negroes, and only rarely in Caucasians, American Indians or Orientals[4]. The distribution of none of these three antigens, however, has been studied widely in Asiatic populations. We consider it worth while, therefore, to present the results of such a study.

Samples were collected in the field into 'Bayer' venules and allowed to clot. They were sent as soon as possible in ice-cold containers to Perth and were generally tested not more than seven days after collection. Adults only were sampled. Although attempts were made to secure blood from unrelated individuals, this was not always possible in the case of relatively small breeding populations, such as the Veddahs in Ceylon, the Irulas and Kurumbas in south India and the Maeo and Yeo tribes in northern Thailand.

All tests were carried out using a standard anti-human globulin reagent prepared at the Commonwealth Serum Laboratories. The reactions were carefully controlled, and only cells were tested which gave a completely negative reaction against the anti-human globulin reagent alone. To check the validity of the Js^a and V reactions cells aged to the same degree were tested with anti-Js^a and anti-V by Dr. Eloise Giblett in Seattle, and known Js^a + ve and V + ve cells sent from Seattle were confirmed by similar reactions in Perth.

The results, as set out in Table 1, show no Js^a + ve or V + ve reactions for 1,274 individuals from twelve ethnic groups in south and south-east Asia. Similarly there were no Di^a + ve individuals among the Veddahs of Ceylon, the Veddoid Irula and Kurumba tribes of South India or among the Tamil, Singhalese, Punjabi or Pathan populations sampled. Di^a + ve reactions were found, however, in Malays, Thais in Bangkok and Chiengmai, the Maeo and Yeo tribes in northern Thailand and the Oraon tribe from the Chota Nagpur Plateau in India. Attention should be directed to the single Indian in the sample from Perlis in northern Malaya who gave a Di^a + ve reaction. This person was born in Malaya, and it is possible that there had been some admixture of Malayan blood which introduced the Di^a antigen in this case.

Table 1. Number of Di^a+ve, Js^a+ve and V+ve Reactions in Blood from Various Populations in South and South-East Asia

Ethnic group	No. tested	Di^a+ve	Js^a+ve	V+ve
Ceylon				
Singhalese	93	*	0	0
Tamils	44	*	0	0
Veddahs	39	0	0	0
India				
Kurumbas	45	0	0	0
Irulas	72	0	0	0
Oraons	125	6	0	0
Pakistan				
Punjabis	168	0	0	0
Pathans	189	0	0	0
Thailand				
Thais (Bangkok)	188	14	0	0
Thais (Chiengmai)	124	9	0	0
Maeo	40	1	0	0
Yeo	26	1	0	0
Malaya				
Malays	40	1	0	0
Indians	131	1	0	0

* Not tested with anti-Di^a.

These results, together with those reporting the absence of Di^a + ve reactions in Australian aborigines and other Pacific Island populations[5], lend support to the view that the Di^a antigen is restricted to people of Mongoloid descent. But if this is true the presence of 5 per cent Di^a + ve individuals among the Oraons of the Chota Nagpur Plateau merits further study. It is possible that there has been a considerable flow of Mongoloid genes into the tribal populations of eastern India, and we hope to publish elsewhere a more detailed analysis of this problem.

The complete absence of the Js^a antigen reactions in the whole of south and south-east Asia, together with its previously reported absence in Caucasian, American Indian and Polynesian populations, supports the view that this antigen is restricted to Negroes. In this connexion its absence in the Irula and

Kurumba tribes, despite the presence among them of a high sickling frequency[4], is of special interest.

Finally, although there has been relatively little study of the distribution of the Rh antigen V, its complete absence in the present investigation is scarcely consistent with the finding of it in low frequency in Oriental, Caucasian and American Indian populations[4]. Because of its low frequency in these populations, however, a much larger series would be needed to exclude its presence in Asian populations with certainty.

We are indebted to Dr. M. Layrisse for supplying anti-Di^a serum and to Dr. Eloise Giblett for supplying anti-Js^a and anti-V sera, and also for assistance in checking Js^a and V reactions. Mrs. E. I. W. Vos gave us valuable technical assistance and many persons assisted in the collection of blood in the field, particularly Drs. R. L. Wickremasinghe, T. Perera, Ali Mohammed Khan, Sommai Sri-ngarm and Sister D. Piljain. The collection of blood samples was made possible by a South East Asia Treaty Organization fellowship to one of us (R. L. K.) and the cost of air transport of samples was defrayed by a grant from the Australian National Health and Medical Research Council. The University of Western Australia also gave generous financial assistance.

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PATHOLOGY

Antibodies against Autologous Brain in Rats with Allergic Encephalomyelitis

It is known that rats and other animals injected with nervous tissue emulsified in Freund's adjuvant may develop allergic encephalomyelitis within 2–3 weeks[1–3]. Many of the injected animals, irrespective of whether they develop encephalomyelopathy, produce complement-fixing antibodies which specifically react *in vitro* with homologous or heterologous brain extracts[4–6]. The role of these complement-fixing anti-brain antibodies, however, in the pathogenesis of allergic encephalomyelitis remains unknown.

Paterson[7] has recently shown that allergic encephalomyelitis may be transferred in rats using lymph node cells from donors injected with nervous tissue-adjuvant. In this work, complement-fixing anti-brain antibodies appeared in the sera of many recipients, whether or not they developed allergic encephalomyelitis. For this reason, it could not be concluded whether the lesions in the recipients were caused by anti-brain antibodies released by the lymph node cells following transfer and/or by the transferred lymph node cells themselves.

Direct evidence that circulating antibodies are important in production of allergic encephalomyelitis, by passively transferring the disease using serum, has not been reported. Attempts by various workers (see review by Chase[8]) to accomplish transfer in this manner have failed. Kabat *et al.*[9] suggested that failure to transfer allergic encephalomyelitis by serum might well be due to continual absorption of circulating antibodies by the donor's own (autologous) brain. Indeed, Kabat, Wolf and Bezer[10] demonstrated that injection of autologous brain can induce allergic encephalomyelitis in monkeys. The present work indicates that complement-fixing antibodies induced in rats by nervous tissue-adjuvant can react *in vitro* with autologous brain.

Each of 22 adult Wistar rats received 0·7 ml. of guinea pig spinal cord homogenate in Freund's complete adjuvant, distributed equally among seven intracutaneous sites over the dorsum and ventral neck. The animals were observed daily for clinical signs of encephalomyelitis (for example, hindleg paralysis) for 21 or 25 days. They were then killed by cardiac puncture to obtain sera for complement-fixing tests. At this time, one half of each rat brain was used for preparation of autologous rat brain antigen. The remaining brain and spinal cord of each animal were used for histological studies. Each rat serum was tested for complement-fixing antibodies against autologous brain and homologous brain and kidney antigens. The complement-fixing technique employed and the method of preparing ethanol extracts of these tissues for use as antigens differed from those used previously[4,11] as follows: (*a*) the tissues were not lyophilized prior to extraction with absolute ethanol; (*b*) the complement-fixing reaction mixtures, containing five 50 per cent hæmolytic units of complement, were held at 4–6° C. for 18 hr. before addition of the hæmolytic system.

Fourteen of 22 sera tested (Table 1) fixed complement with autologous brain. Eleven of these 14 sera also fixed complement with homologous brain. None of the remaining 8 of the 22 sera tested fixed appreciable amounts of complement with either autologous or homologous brain. Past work in our laboratory indicated that such complement-fixing antibodies are not present in sera of normal rats or in sera of rats injected with guinea pig kidney-adjuvant[12]. Those sera tested against homologous kidney were negative.

Table 1. Complement-fixing (CF) Antibodies in Sera of Rats after Injection of Guinea Pig Spinal Cord in Freund's Adjuvant

Rat No.	CF antibody titre of sera against indicated antigen*		
	Autologous brain	Homologous brain	Homologous kidney
*D*321	8†	8	< 4
*D*322	16	32	< 4
*D*325	32	32	< 4
*D*331	4	4	< 4
*D*333	16	8	< 4
*D*334	16	< 4	ND‡
*D*335	8	8	< 4
*D*385	8	4	ND
*D*387	4	< 4	ND
*D*388	8	4	ND
*D*390	8	4	ND
*D*392	8	4	ND
*D*394	8	4	ND
*D*396	4	< 4	ND

* Sera tested in dilutions of 1-4 to 1-128 against 1 : 25 dilution of brain and kidney antigens.

† Reciprocal of greatest dilution of serum giving maximum fixation of complement.

‡ ND, not done.

As expected from previous work[4-6,12], no direct correlation existed between the occurrence of allergic encephalomyelitis and the presence of the complement-fixing antibodies detected here against either autologous or homologous brain in individual rats. Ten of 14 rats, the sera of which reacted with autologous brain, had clinical signs and/or lesions of allergic encephalomyelitis. Five of 8 rats, the sera of which did not react with autologous brain, developed disease.

Our results (Table 1) indicate that injection of rats with heterologous spinal cord may induce complement-fixing antibodies which react *in vitro* with autologous nervous tissue.

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Tyrosyluria in Human Subjects

Tyrosyluria, that is, an abnormal excretion of aromatic metabolites of tyrosine, has long been known to occur in human subjects in ascorbic acid deficiency, particularly after a high tyrosine diet; it is readily abolished by feeding ascorbic acid. Recently, however, Bloxam *et al.*[1] have reported fairly rare cases of a tyrosyluria in apparently normal infants which did not seem to result from ascorbic acid-deficiency and which disappeared spontaneously with time.

We find this observation particularly interesting, since we believe that other cases of tyrosyluria, detectable by chromatographic methods though probably mostly within the normal range of less-specific analyses, occur frequently in ill subjects. We have previously described an increased excretion of some tyrosine derivatives in stress[2], and have now often observed similar phenomena in pathological urines of many types, from subjects not obviously suffering from lack of dietary ascorbic acid or from liver damage.

In many of these subjects, as with those of other workers, the characteristic compounds excreted in abnormal quantity are *p*-hydroxyphenyl-pyruvic and -lactic acids. Both substances are probably invariable minor constituents of normal human urine: the pyruvic acid has always been detected (as *p*-hydroxybenzalhydantoin) in extracts of acid-hydrolysed urine[3] and is presumably accompanied by trace amounts of the lactic acid, its reduction product. However, using extracts of urine equivalent to 1-min. excretion prepared by the method of Hill *et al.*[4] and chromatographed in *iso*propanol–ammonia followed by *n*-butanol–pyridine–water (70 : 10 : 20), we have but rarely detected (diazotized sulphanilic acid) *p*-hydroxyphenyllactic acid in healthy male British subjects, mostly young Royal Air Force personnel. It may be noted, however, that Armstrong *et al.*[5] reported it as being one of the more common constituents in their normal urines. Though this difference from our observations may well be accountable in terms of technique, diet and a less-restricted age-group of subjects, at least one other factor is of interest—the altitude (5,000 ft.) of the Salt Lake City laboratories of Armstrong and his group. Tyrosine metabolism is so linked with that of ascorbic acid that possible changes in the latter as a result of anoxia[6] leaves some ground for speculation.

Bloxam *et al.*[1] noted that the urines they examined contained *p*-hydroxyphenylacetic acid, while Boscott and Cooke[7] examined a group of subjects who rarely excreted *p*-hydroxyphenyllactic acid, but in whom the major tyrosine metabolite appeared to be *p*-hydroxyphenylacetic acid. The technique employed by the latter workers is open to some criticism, while the former did not comment on the excretion of the acetic acid by infants in general and made no claims regarding its significance. Further difficulties in interpreting this latter arise, since the acetic acid might originate from tyrosine via tyramine or via *p*-hydroxyphenylpyruvic acid, either metabolically or by decomposition in urine. At least in rats, recent work[8] has shown that it does not appear to be dependent on intestinal micro-organisms. We find *p*-hydroxyphenylacetic acid to be invariably detectable in normal urine (cf. ref. 5). Occasionally it has been detected in usually large quantity, but, with the exception of one subject who excreted it consistently thus, such occasions appear to be atypical of the subjects. In contrast, high rates of excretion have often been noted in both stress and other pathological conditions, not necessarily in subjects also excreting *p*-hydroxyphenyllactic acid. It seems necessary to consider the possibility that two distinct types of tyrosyluria do in fact exist.

Yet other tyrosine derivatives are sometimes present in large quantity in pathological urines: *p*-hydroxyphenylpropionic acid has only occasionally been noted; *p*-hydroxybenzoic acid is unfortunately certainly only derived in part from tyrosine; tyramine may be intimately connected with *p*-hydroxyphenylacetic acid; the compound recently identified with *p*-sympatol[9] may, by reason of its known physiological activity and unusual structure, be more than a mere by-product of tyrosine degradation. Another type of hydroxyphenyluria is characterized by the excretion of a group of unidentified phenols, sometimes in such relatively large amounts that it is difficult to account for them except in terms of tyrosine metabolism (the possibility of an origin from drugs has been excluded). Failure to react with 2 : 6-dichloroquinonechloroimide indicates the presence of the appropriate *p*-hydroxy grouping, while ease of oxidation suggests a possible second oxygen substituent (perhaps a conjugated hydroxyl group) in the benzene nucleus. It is of interest that Bloxam *et al.*[1] detected an unknown phenol, while Boscott and Cooke[7] more specifically stated that their subjects excreted large quantities of polyphenols, including catechol. Further, in the single classical case of 'tyrosinosis'[10] the subject excreted, besides *p*-hydroxyphenyl-lactic and -pyruvic acids, a third compound identified as 3 : 4-dihydroxyphenylalanine, though unfortunately only on the basis of its properties and a carbon–hydrogen analysis. Normally degradation of tyrosine proceeds through a 2 : 5-dihydroxyphenol, homogentisic acid.

The term tyrosyluria may cover a number of abnormalities: how each may best be characterized is at present problematical. It seems likely that the development of accurate quantitative analyses for many urinary phenols will be essential for further progress.

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RADIOBIOLOGY

Strontium-90 in Human Plasma

THE average daily dietary intake of strontium-90 in Britain during 1958 has been assessed as about 6 μμc. per gm. calcium[1]. It is well known that discrimination against strontium relative to calcium takes place in absorption from the gut and excretion by the kidney[2]. The combined effect of these discriminatory processes would lead to an expected concentration of strontium-90 relative to calcium in human plasma of about one-quarter that in the diet, that is, about 1·5 μμc. strontium per gm. calcium in 1958. No measurements of the strontium-90 content of human plasma have yet been reported. On the basis of the above estimation, at least 20 litres would be required for a measurable activity to be obtained. Thus the same individual must be bled on a large number of occasions over a long period of time or many individuals must be bled to provide a bulked specimen.

The Blood Products Laboratory, Lister Institute, provided us with 70 bottles containing about 120 litres of pooled plasma unfit for transfusion because of bacterial contamination. This had been prepared from whole blood collected between November 1958 and March 1959, except for about 2 litres dating from mid-1958. The consignment was divided into two parts: (1) South London, Birmingham, Bristol, Cambridge, Cardiff, Leeds and Belfast; (2) Liverpool, Manchester, Newcastle, Sheffield and the north-east Metropolitan area. The strontium-90 content per gm. calcium in these two bulked plasmas has been determined. The results are taken to be representative of an adult population at the end of 1958.

Strontium together with calcium was precipitated in each bottle of plasma by the addition of a known excess of saturated oxalic acid. The solids were separated by centrifuging, bulked and thermally ashed at about 400° C.

The calcium content of each of the two batches was determined on a weighed aliquot by the standard oxalate – permanganate method. Strontium was separated from the large excess of calcium in the resulting ash by successive precipitations of strontium nitrate in 78–80 per cent nitric acid. The strontium-90 content was determined by separating chemically the daughter product, yttrium-90, after radioactive equilibrium had been re-established. The radioactive decay of yttrium-90 (half-life, 64 hr.) was followed over about eight days using an anti-coincidence counter. After correction for chemical loss (about 40 per cent) and self-absorption (5–10 per cent) the final results obtained were 1·1 and 1·4 μμc. strontium-90 per gm. calcium in the two batches. The estimated error in each observation was 25 per cent, so that no significance is attached to the difference between the two results. The observed concentrations of strontium-90 in plasma are thus consistent with a daily dietary intake of 6 μμc. per gm. calcium

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Strontium-90 and Cæsium-137 in Grass and Milk in Finland during 1959

MILK and milk products are the main source of calcium (85 per cent of the total) in the average Finnish diet. As they are also important links in the transfer of radionuclides from atmospheric fall-out to the human body, we have started an investigation to determine the contents of some important radionuclides in Finnish milk. Results of determinations of strontium-90 and cæsium-137 for the year 1959 are reported here.

Two sets of samples were collected. 'Local' samples (Nos. 1–9, Table 1) were taken during the period August 29–September 6, 1959, on nine experimental farms between latitudes 60–69° N. and longitudes 23–27° E., where soil conditions, fertilizer practices and other agricultural factors are known in detail. 'General' samples (Nos. 10–15), analysed for strontium-90 only, were taken between May 1 and November 17, 1959, from one-day's milk intake (60–80,000 litres) of two milk-powder factories of the Kuivamaito Oy, located in south-east (*A*) and south-west (*B*) Finland. Both factories collect milk from more than ten dairies within a radius of 100 km. between latitudes 60° and 62° N.

Representative samples of grass were also collected from the experimental farms for analysis and determination of the discrimination factors for grass and milk of strontium-90 and cæsium-137.

Table 1. Calcium, Strontium-90 and Cæsium-137 Contents of Grass and Milk Samples from Finland (Mean Values of Duplicate Determinations)

Sample				Calcium/gm. ash		μμc. Strontium-90/gm. ash		μμc. Strontium-90/kgm. dry weight		μμc. Strontium-90/gm. calcium*		μμc. Cæsium-137/gm. potassium†	
No.	Origin	N. Lat.	Date (1959)	Grass	Milk	Grass	Milk	Grass	Milk	Grass	Milk	Grass	Milk
				Experimental farms :									
1	Apukka	66°	Aug. 29	7 46	16 5	7·19	0 61	632	35 2	96	3 7	87	60
2	Viik	60°	Aug. 29	6·54	15 5	5·77	1 00	594	55·4	89	6 7	220	43
3	Malminkartano	60°	Aug. 31	5·89	17 5	7 45	0·79	690	45 0	126	4 5	98	37
4,1	Halola	63°	Sept. 1	4·21	16·2	5·21	0 67	517	35 3	122	4·2	—	
4,2	Halola	63°	Sept. 1	6 39		5·75		570		92		—	21
5	Jokioinen	61°	Aug. 30	7 85	14 3	6·43	0·52	528	30·0	82	3 6	86	21
6	Anttila	60°	Aug. 31	6 59	16 0	5·09	0 78	635	43 7	77	4 9	290	25
7,1	Kreuksela	65°	Sept. 2	4·23	16 8	5·77	1 12	704	61 6	138	6 6	210	51
7,2	Kreuksela	65°	Sept. 2	4·78		5·90		661		123		190	
8	Myttaalä	61°	Aug. 29	3·74	16·6	6 61	0·98	609	53 3	177	5 9	100	19
9	Toivonniemi	69°	Sept. 6	9 28	17 3	20·83	2 01	1,581	96 5	224	11·6	1,100	220
				Milk-powder factories :									
10	Kuivamaito Oy, *A*	60–61°	May 1		16·5		1 61		118		9 8		
11	Kuivamaito Oy, *A*	60–61°	June 28		16 8		1 63		125		9 7		
12	Kuivamaito Oy, *A*	60–61°	Aug. 15		16 4		1 31		96 3		8·0		
13	Kuivamaito Oy, *A*	60–61°	Nov. 17		17·4		1 47		118		8 4		
14	Kuivamaito Oy, *B*	60–61°	May 30		17 1		1 36		106		7·9		
15	Kuivamaito Oy, *B*	60–61°	June 1		16 5		1·41		109		8 6		

* The error due to counting (two standard deviations) is < 14 per cent.
† Standard deviation less than 20 per cent.

Radiostrontium was isolated by the method of Bryant *et al.*[1] and β-activity was determined with a low-background counter *CE*-14 manufactured by Tracerlab.

Cæsium-137 was determined γ-spectrometrically in milk and grass ash prepared below 450° C. in the manner described, for example, by Löw and Edvarson[2] and by Lillegraven[3]. The shielding system and pulse height analyser used in this work have been described by Vuorinen[4]. The cæsium-137 activity of the milk-ash samples was calculated after correction for background and potassium counts.

Determination of cæsium-137 in the grass-ash samples was only possible after an estimation of the relative amount of zirconium-95 + niobium-95 in the spectrum (Fig. 1) by using the channels outside the maximum of the cæsium photopeak (region I in Fig. 1). A correction for zirconium-95 + niobium-95 pulses in the cæsium-137 channels, in addition to the background and potassium-40 pulses, thus became possible.

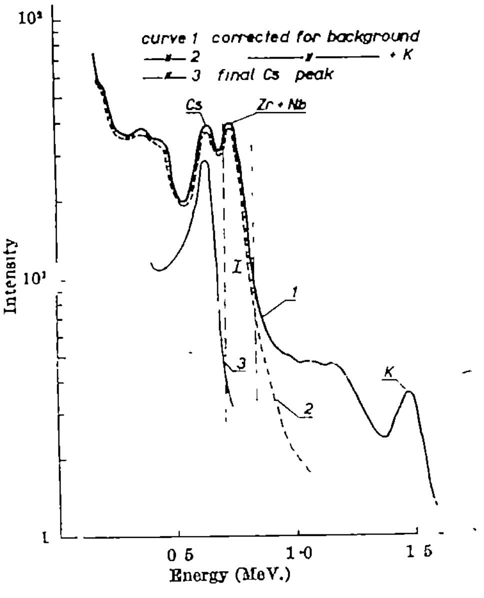

Fig 1. Differential pulse-height spectrum of the grass sample No. 9

The results are presented in Table 1. They show that the strontium-90 and cæsium-137 contents of milk in Finland do not differ essentially from that of the milk of other countries in corresponding climatic conditions[5,6]. The discrimination factor for grass and milk, that is, the ratio μμc. strontium-90/gm. calcium in milk divided by this ratio for grass, is about 0 048. The corresponding factor for cæsium-137 is 0·28.

The ratio μμc. cæsium-137/gm. potassium per μμc. strontium-90/gm. calcium for grass is 1·95 : in milk the mean for the samples from south and middle Finland (Nos. 2–8) is 5·66. The values of the two Lapland samples, however, are significantly higher, namely, the sample from Apukka (No. 1) 16·2 and that from Toivonniemi (No. 9) 19·0.

More detailed reports on this investigation will appear elsewhere.

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Glutamic Acid Metabolism in Brain and Liver during Infusion with Ammonia labelled with Nitrogen-15

RECENT results of *in vivo* experiments using glutamic acid labelled with carbon-14 were consistent with the hypothesis that the amidation of the administered amino-acid to glutamine represents a metabolic event occurring in a tissue or cellular compartment[1,2]. Since glutamic acid and glutamine syntheses in the central nervous system are the two known processes by which ammonia enters organic linkage, it appeared of great interest to approach the question of setting into compartments the metabolism of glutamic acid and its derivatives with the aid of ammonia labelled with nitrogen-15.

^{15}N-ammonium acetate was infused into the carotid artery of cats over a period of 8–85 min. with simultaneous electroencephalographic and electrocardiographic tracings. The experiments were terminated by exsanguination of the animal, the blood was collected, brain and liver excised and frozen, and the level of glutamic acid and its metabolic derivatives determined in the tissue samples[3,4] as well as the concentration of nitrogen-15 in the amino or amide groups respectively of these compounds. The results of a typical experiment of 25-min duration are given in Table 1.

Table 1. ISOTOPIC CONCENTRATION IN GLUTAMIC ACID AND RELATED METABOLITES AFTER NITROGEN-15 LABELLED AMMONIA INFUSION INTO THE CAT

	Blood		Brain cortex		Liver	
	μM/gm. tissue	N-15 Atom per cent excess	μM/gm. tissue	N-15 Atom per cent excess	μM/gm. tissue	N-15 Atom per cent excess
Ammonia	2·8	81·9	1·8	65·7	4·6	42·6
Glutamine amide	0·32	21·4	7·8	38·7	0·83	28·7
Glutamine α-amino	0·32	1·8	7·8	8·6	0·83	5·4
Glutamic acid	0·08	3·1	9·8	0·87	1·6	13·6

Nitrogen-15 ammonium acetate (99·6 atom per cent excess) 1 mM/ml. infused into carotid artery of a succinylcholine paralysed 3·3 kgm. cat at a uniform rate for 24 min. (total dose, 16 m.moles). Experiment terminated at 25 min. by bleeding from the carotid artery; cortex and liver frozen *in situ* with solid carbon dioxide.

While the concentrations of the amino-acids measured in the various tissues remained constant or decreased slightly, the level of glutamine in the brain increased by at least 50 per cent (for normal values see ref. 4). The observation that the nitrogen-15 content of the amide group of cerebral glutamine is higher than that of liver or blood indicates that this amide is synthesized in the brain. Since the level of glutamine in brain increased considerably, the high specific activity of the amide group is probably not due to an exchange with free ammonia but to a net synthesis of glutamine. Circumstantial evidence for glutamine synthesis in the brain has come from experiments on hepatectomized animals[5] and is consistent with the high synthetase activity in this organ[6,7].

Of particular interest is a comparison of the concentration of nitrogen-15 in the α-amino group of glutamic acid and of glutamine isolated from the brain. Since the α-amino group of glutamine has ten times the specific activity found in glutamic acid, brain glutamine must be derived from a compartment of glutamic acid which is not in equilibrium with the total tissue content of this acid unless glutamine is synthesized appreciably by a pathway which does not require glutamic acid as the immediate precursor.

In contrast to the observations on brain, the α-amino group of liver glutamine is of lower specific activity than that of liver glutamic acid, a result which might be expected if the glutamic acid used for glutamine synthesis in the liver mixes rapidly with the total tissue pool of this amino-acid. However, in preliminary double-labelling experiments (^{14}C glutamic acid and $^{15}NH_3$) compartmentalization of glutamic acid used for glutamine synthesis can also be demonstrated in this organ in the presence of high ammonia concentrations.

If glutamic acid is utilized for the synthesis of the additional glutamine in the brain, the former amino-acid is apparently made in this organ and not derived from the blood, since the nitrogen-15 content of the α-amino group of brain glutamine (8·6 per cent) is higher than that of blood glutamic acid (3·1 per cent).

According to present evidence the reductive amination of keto-glutaric acid occurs in the mitochondria[8] while glutamine synthetase is localized in microsomes and mitochondria[9]. Further experiments will have to show whether the results of this and previous work together with evidence for the enzyme distribution in the cell are indicative of different metabolic pools within the cells for the metabolism of administered glutamic acid and of that synthesized in the tissue. This type of investigation may point the way by which the problem of setting into compartments of metabolic events *in vivo* may be approached[10]. A detailed account of the experiments as well as of the effects of ammonia infusion will be given elsewhere.

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Alpha-Activity of Wheat and Flour

IN their work on naturally occurring radioactivity in man and his environment, Mayneord and his co-workers have indicated[1,2] the wide range of alpha-radioactivity found in the more common foodstuffs. Using the same experimental method, we have recently made measurements on a number of samples of wheat and wheat products of known origin, and wish in this communication to report briefly on three aspects of our findings.

Using wheat from the United Kingdom, Canada, New Zealand, Victoria, South Australia and Western Australia, we have noticed that, while there are typical differences between wheats from these sources, with wheat from any one source there appears to be a connexion between alpha-activity and soil *p*H. As an example, we are indebted to the National Institute of Agricultural Botany for a series of samples of wheat varieties grown together, in trial, at nine different centres. A relatively small range in activity occurs between varieties, but as between locations we found these limiting cases: variety Cappelle Desprez grown on chalk soil at Sparsholt in Hampshire had an activity of 0·21 μμc./gm. ash, soil *p*H 7·8; the same variety grown on an acid loam at Winnarleigh in Lancashire had an activity of 1·66 μμc./gm. ash, soil *p*H 5·2. This ratio of 8 : 1 contrasts with that of only 2·5 : 1 for variety; the highest activity found at Sparsholt occurred in the variety Dominator, which was measured as 0·49 μμc./gm. ash. This effect of soil *p*H was also found in all foreign wheats when variety samples were compared.

With milled wheat we have compared the activities of bran and white flour with the wheat from which they were derived. The mean of six samples in each case was: wheat, 1·1 μμc./gm. ash; bran, 1·0 μμc./gm. ash; flour, 1·0 μμc./gm. ash.

It would thus appear that the alpha-activity of wheat is of soil origin, in contrast to the occurrence of strontium-90, in which the much higher content in bran as compared with flour has been interpreted[2] as due to retention of air- or rain-borne material in the developing ear of the plant.

In wheat from widely separated sources we have observed a considerable range in alpha-activity:

Source of wheat	Samples tested	Mean activity (μμc./gm. ash)
United Kingdom	30	1·0
Canada	15	1·0
New Zealand	30	2·5
Victoria	15	2·5
South Australia	2	2·6
Western Australia (Fremantle)	3	25·0

In respect of the high figures for Western Australian wheat, consistently high activities were obtained from three different shipments of this grain. The ratios of activity from thorium and radium families were similar in all cases, averaging 30 per cent.

Finally, we would mention that these results might be better expressed in terms of the calcium with which they are associated. Wheat ash (1·4–1·8 per cent of the dry weight) contains about 2·8 per cent of calcium, whereas in white flour the ash (0·5 per cent dry weight) contains nearly 4 per cent. Present legislation, however, makes it compulsory in the United Kingdom to add, as creta praeparata, 0·125 per cent of calcium to flour (except to wholemeal), thus increasing its content of calcium to 0·145 per cent.

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BIOLOGY

Use of Gibberellin to hasten Germination of *Solanum* Seed

True seed of the potato (*Solanum tuberosum* L.) frequently has a delayed germination[1], and we find that this also applies to most other tuber-bearing *Solanum* species, where it is usual for seed to remain dormant from 1–3 months or longer. Geneticists and potato breeders have been inconvenienced by this difficulty. Our results with certain techniques[2,3] for hastening germination of *Solanum* seed have been variable. Since gibberellin has been used to promote germination and terminate dormancy of seeds in other genera[4–6], its effectiveness to hasten germination of *Solanum* seed was investigated by us.

In preliminary tests, gibberellin concentrations of up to 100 p.p.m. had little effect on breaking dormancy of recently harvested *Solanum* seed, but germination was stimulated by concentrations of 1,000–4,000 p.p.m. To confirm this result, dry seeds of several *Solanum* species and species hybrids were examined microscopically with transmitted light to select those containing mature embryos. Lots of 50 seed were soaked for 24 hr. in different concentrations of the potassium salt of gibberellic acid (75 per cent actual). The seeds were then washed in three successive changes of distilled water and placed on moist blotting paper in Petri dishes kept at room temperature (20–30° C.). As controls, similar lots of seed were treated with distilled water but otherwise handled in the same manner. The results (Table 1) show per cent germination for gibberellin-treated seed.

Although prolonged exposure of the seeds to the 1,000 p.p.m. concentration increased per cent germination appreciably, treatment with 2,000 p.p.m. for

Table 1. Effect of Gibberellin on Germination of Recently Harvested *Solanum* Seed

Solanum species		Age of seed in days after harvest	Germination (per cent)*			
			Concentration of gibberellin (p.p.m.) 0	1,000	2,000	4,000
S. chacoense Bitt., clone *A*	*SC*†	8	52/60	—	90/96	92/100
S. chacoense Bitt., (clone *A*) × *S. emmeae* Juz. et Buk.	—	17	14/18	—	60/84	78/94
S. demissum Lindl., clone	*S*‡	1	0/2	58/84	90/100	96/98
S. jamesii Torr.	*SC*	7	0/0	—	36/40	—
S. pinnatisectum Dun. × *S. jamesii* Torr.	—	4	0/6	—	28/78	—
S. phureja Juz. et Buk., clone	*SC*	2	50/74	—	98/98	—
S. stoloniferum Schlechtd., clone *A*	*S*	8	0/10	100/100	—	—
S. stoloniferum Schlechtd., clone *B*	*S*	1	0/0	—	32/100	—
S. trifidum Correll × *S. jamesii* Torr.	*S*	1	28/46	—	86/98	—
S. tuberosum L., variety Hunkuil	*OP*§	8	0/8	4/58	—	—
S. tuberosum L., variety Hunkuil	*OP*	60	4/38	40/84	60/96	38/94
S. tuberosum L., variety Katahdin	*S*	14	8/10	—	96/98	—
S. tuberosum L., *F* 4792	*OP*	60	26/68	100/100	98/98	96/96
S. verrucosum Schlechtd.	*SC*	7	0/0	—	0/46	—

* Fifty seeds were soaked in the gibberellin solutions for 24 hr. Numerator is the percentage of germination in 15 days, denominator is that in 30 days.

† Sibling cross.

‡ Selfed.

§ Open pollinated.

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24 hr. gave optimum germination. The 4,000 p.p.m. concentration was no more effective than the 2,000 p.p.m. concentration. At the optimum concentration the majority of treated seeds had germinated in 15 days. The gibberellin treatment accelerated germination in seeds which had little dormancy and in seeds approaching the end of their dormancy period. Seeds of *S. demissum* Lindl., U.S.D.A., *P.I.* 160227 and of a *S. chacoense* Bitt. × *S. phureja* Juz. et Buk. derivative exhibited no dormancy. Similarly, Odland[7] reported immediate germination in a number of seed lots of *S. tuberosum*.

Plants were grown to maturity from the gibberellin-treated seeds, and in all cases growth was normal. The above method has been used in our work with *Solanum* species and allows us to grow many more plant generations in a given period of time.

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Pollen Germination in Some Gramineae

I. K. Vasil, in his communication under this title in *Nature* of September 24, p. 1135, states that "this is the first report of a successful germination of the pollen of Gramineae *in vitro*". May I point out that the germination of pollen of different varieties of *Saccharum officinarum* was carried out as a routine practice by the Sugarcane Research Station in Mauritius as far back as 1930. The methods used are described by Glendon Hill[1] and de Sornay[2]. Germination tests were discontinued later, as it was found that there was poor correlation between germination of pollen *in vitro* and number of sugar cane seedlings afterwards produced[3].

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A Selective Medium for the Formation of Ascospores by *Aspergillus nidulans*

Little is known about the ascospore composition of the Ascomycetes, partly because of the difficulty of obtaining a massive production of such cells. During an investigation of the nutritional requirements of a strain of *Aspergillus nidulans* obtained from the Botany School Collection, Cambridge, observations were made that led to the recent development in this laboratory of a method for obtaining satisfactory yields of ascospores without interference from the conidiospores. This fact has enabled fractionation studies on *A. nidulans* ascospores to be undertaken, as well as other biochemica investigations which are now in progress.

Microscopically and culturally the strain used in these studies is similar to that described by Thom and Raper[1]. This organism, when cultured in Czapek's agar, produced colonies characterized by a dense vegetative mycelium spreading quickly on the agar, with abundant green conidial heads freely distributed on the surface of the colony, and relatively few perithecia scattered through the different area of the colony; ascospore production lasted severa weeks.

When the mould was cultured on a medium with the following composition: Urea or ammonium oxalate, 3 gm.; dipotassium hydrogen phosphate 1 gm.; crystalline magnesium sulphate, 0·5 gm. potassium chloride, 0·5 gm.; ferric sulphate, 0·05 gm.; sucrose, 30 gm.; agar-agar, 15 gm., and distilled water, 1,000 ml., colonies were formed with very different general morphology from those obtained on Czapek's medium. Under these conditions perithecia developed separately within or on the conidial layer with a dark reddish purple coloured envelope of scattered hyphæ bearing a considerable number of Hulle cells. On ripening, these became a mass of eight-spored asci which broke down quickly, releasing the ascospores. When a preparation of this material was examined under the microscope an enormous number of purple-red lenticular ascospores, with two equatorial crests, were observed, with very few contaminating conidiospores.

Another characteristic was particularly marked when plates were viewed in reverse, namely, an intense purple pigmentation extended through all the medium marking the under-surface of perithecia and ascospores. This pigmentation was absent after growth on plain Czapek's agar.

We are indebted to Mr. J. Bean of the Botany School, University of Cambridge, for sending us a culture of this mould.

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Degeneration of Flight-musculature in the Corixidae and Notonectidae

Within the New Zealand species of the families Corixidae and Notonectidae (Hemiptera-Heteroptera), two distinct forms, depending on the development of the wings and the wing-musculature, have been recognized. In these species a proportion of the population is composed of flightless animals in which, apart from *Diaprepocoris zealandiae*, the indirect flight-musculature is reduced even though the two sets of wings are not significantly altered from the normal condition. In *D. zealandiae* the meta-wings are greatly reduced and the meso-wings differ in having a reduced membranous portion.

The differences in the musculature are obvious immediately the mesothoracic tergite is removed, for in place of the solid blocks of yellow fibre the flightless form contains either a pale white, loosely compacted ibrous mass, or, more usually, only parenchymatous tissue. There are a number of differences, too, in the form of the body and the extent of chitinization and pigmentation. Without recourse to dissection, the two forms may be readily distinguished. Flightless animals, besides being significantly smaller, are less pigmented, and the chitinization, particularly of the thorax, is greatly reduced.

Although the degree to which the flightless animal is modified from the normal condition varies throughout these species, within each of them the extent of the modification is remarkably uniform and no intermediate forms have been discovered. This uniformity applies not only to the condition of the musculature but also to the modifications already noted. This situation follows from the mechanism by which the condition is developed. Normal growth of the wing-musculature is not continued throughout the fifth instar, and at the conclusion of the moult to the imago the final modifications of the skeleton have been achieved, although the histolysis of the partly developed fibres and their replacement by parenchyma continues for a short period.

The factors responsible for the control of the development of the muscle-form reside in the environment, and until late fourth instar either form may be produced depending on the habitat conditions. A close association between the environmental conditions, with respect to stability of the habitat during drought, and the muscle-form developed in the population, results from delaying the critical period for this control until the fifth instar. Almost without exception, the less-stable habitats within the species range contain only animals with normal musculature. In stable areas—streams and lakes in which the population may be maintained throughout the year—both forms are found, but the population is predominantly of flightless individuals. Flying animals are necessary for the maintenance of populations in the unstable habitats of the species range; through migration they re-establish the species in habitats that have been dry during the summer.

From a comparison of the extent of the changes that are associated with the cessation of wing-muscle growth in the species of *Sigara* and in *D. zealandiae*, it is apparent that the loss of this musculature is an early stage in the process leading to the brachypterous condition: the wing-musculature is first reduced and only then are the meta-wings modified.

In the New Zealand fauna this condition of the musculature which gives rise to flightless animals without alteration of either pair of wings has been recognized in each of the seven species of *Sigara* (*Tropocorixa*) and in the two species of *Anisops*. With experience of the modifications resulting from the change in musculature, it is apparent from the accounts of these two families in other localities that this condition occurs in a number of other species. The two colour forms described by Brooks[1] in *Anisops* are due to this feature. *A. wakefieldi*, a species found in New Zealand, is considered in this work and the two forms dependent on the condition of the musculature are described, although no explanation is offered to account for them. The numerous brachypterous forms recorded in the species considered by Hungerford[2] and Poisson[3] suggest that many other species in which the musculature only is reduced probably occur also. Finally, Brown[4] attributes the anomalies noticed between the abundance and migration-rate in a series of British Corixidae to physiological factors. It seems more likely that, as the species in which the relation was least satisfactory were associated with stable habitats, this was a consequence of the reduction of the flight musculature in these animals.

A fuller account of this work is being published elsewhere.

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Environment and Reproduction in Freshwater Triclads

Reproduction in triclads varies from asexual to complicated sexual methods[1,2] both within and between species. Attempts to determine the causes of such differences resulted in controversy between students favouring the external environment[3] and those favouring an internal physiological process[4,5] as the determinant.

Dahm's[2] researches on triclads inhabiting running water, coupled with work[6,7] on the triclads of standing water, suggest a broad relationship between habitat and mode of reproduction. When triclad species are arranged in a series based on increasing productivity of the habitat (*sensu* Ruttner[8]), often accompanied by higher average temperatures, it extends from *Phagocata vitta*, which is found in the harshest conditions inhabiting moorland streams and upland lakes, to *Dugesia lugubris*, which is found only in the most productive lakes and quieter reaches of some lowland rivers. Table 1 shows such a series for the commoner freshwater triclads of northern Europe

Table 1. North European Species arranged in Order of Productivity of their Habitats, showing Prevalent Types of Reproduction

	Species	Habitat		Reproduction			Cocoons
		Lotic	Lentic	Asexual only	Both	Sexual only	
Increasing productivity ↓	*Phagocata vitta* (Dugès)	X		+++	+	+	Winter
	Crenobia alpina (Dana)	X		+++	++	+	Winter
	Polycelis felina (Dalyell)	X		+++	+	+	Winter
	Dugesia gonocephala (Dugès)	X		+	+	+++	—
	P. nigra (Mull.)	X	X		+	+++	Early spring
	P. hepta (E. H. and Y. Melander)		X			+++	Spring
	P. tenuis (Ijima)		X			+++	Spring
	Dugesia lugubris (Schmidt)		X			+++	Early summer
	Dendrocoelum lacteum (Mull.)		X			+++	?

together with the mode of reproduction and the season of sexual reproduction.

Asexual reproduction is the usual means of population increase in species occupying unproductive habitats, and it becomes progressively less important and is finally replaced altogether by the sexual process in species found in the most productive habitats. This is also accompanied by a change in the season at which the sexual process occurs. Thus, in *Phagocata vitta* and *Crenobia alpina*, and to a lesser degree in *Polycelis felina*, sexual reproduction occurs mainly in winter in North European populations. *Polycelis nigra* starts to breed in the early spring[9], *P. tenuis* and *P. hepta* slightly later, while *D. lugubris* does not begin until the late spring or early summer[10].

Dahm showed that growth and reproduction of species living in unproductive streams are possible only when the basic metabolism is slowed down by low temperatures characteristic of winter conditions; this applied over a range of feeding regimes. In species such as *P. tenuis* which live in lakes of intermediate productivity I have found that, while winter temperatures inhibit breeding, it occurs at temperatures characteristic of the British spring and summer. Breeding in *D. lugubris* is rather more sensitive to low temperatures and rather less to high temperatures. In both species breeding ceases when they are kept at temperatures higher than those of an average summer (about 24° C.), and this is followed by retrogression of the reproductive organs and a decline in size despite a plentiful supply of food. Such a relationship between energy intake and expenditure might explain the infrequency of sexual reproduction in species occupying unproductive habitats and its restriction to colder periods. Under such conditions food would never be plentiful, but would be particularly scarce when low temperature would favour the channelling of energy into growth and ultimately reproduction. Sexual reproduction therefore might be so restricted that without other means of multiplication many populations would become extinct. The existence of asexual methods of reproduction in such species as *Ph. vitta*, *C. alpina* and *P. felina* solves this problem. The tendency to breed in winter suggests that high temperature is more important than scarcity of food in preventing sexual reproduction under natural conditions in northern Europe. In contrast, sexual reproduction in species living in productive habitats would be possible each year when abundance of food (relative to activity), permitted growth and reproduction in a proportion of the population. In these circumstances sexual reproduction, which is regarded as conferring advantages from an evolutionary point of view, would be more common.

Clearly, apart from the basic differences in the ability of the several species to function at various temperatures, any differential effect of temperature might emphasize the contrasts outlined above between the triclads of unproductive and productive habitats. From the available data[11,12] such a differential appears to be present, and species like *C. alpina* and *P. felina* not only have a higher basic level of activity but also are unable to control their activity when the temperature is increased as, for example, *Dugesia gonocephala* can.

It is interesting to note that Dahm also found that within a single species there were populations which could only reproduce asexually (usually polyploids with odd multiples of the haploid chromosome number), some which could multiply both sexually and asexually, and others which showed only sexual reproduction. It would be rewarding to study the ecology of such populations to determine the relationship between the habitat and mode of reproduction.

If it is assumed that the primitive triclad reproduced by both methods, then it appears that selection has operated to suppress sexual reproduction in the species of unproductive habitats and asexual reproduction in those of productive habitats.

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A Possible Method of Sex Ratio Determination in the Parasitic Hymenopteran *Nasonia vitripennis*

Nasonia vitripennis (Walter) (Pteromalidae) is parasitic on the pupal stage of muscoid flies. The female drills through the host puparium and lays eggs on the pupa within. When a population of *N. vitripennis* has ready access to host puparia, each generation of the offspring is composed of females and males in the ratio 3 : 1[1]. The females are diploid and the males haploid[2]. So far, no suggestion has been made as to how this sex ratio may be determined; but it seems possible that it may be affected by the arrangement of the eggs in the ovariole before fertilization. The eggs are shaped like curved sausages and are more pointed at one end than the other. This is the typical hymenopterous shape. Owing to its curvature, each egg has in its meridional plane a convex and concave side. Within each ovariole the eggs are orientated so that the oldest eggs, namely those nearest the oviduct end of each of the four ovarioles which comprise a single ovary, almost always have their convex aspect pointing away from the axis of the ovary as a whole (Fig. 1). This arrangement was observed in 46 out of 50 insects examined.

The chorion of the egg is rigid compared with the very thin walls of the vagina and oviduct, and it is a reasonable assumption that the eggs either maintain their orientation or twist in a more or less constant manner as they leave the ovariole and pass one at a time through the short oviduct into the vagina. If either of these suppositions were true, the eggs from adjacent ovarioles would lie in the vagina successively so that their concave aspects make an angle of 90° to each other. All the eggs from any one ovariole should generally be orientated the same way when they arrive in the vagina.

In the vagina, the eggs come into contact with a muscular tongue which projects into the vagina from

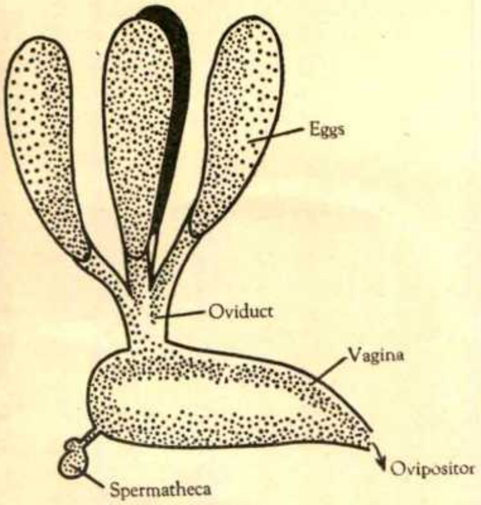

Fig. 1

its dorsal surface (Fig. 2). Flanders[3] described a similar tongue in *Nemeritis canescens* (Grav.) and suggested that it might press the micropyle of the egg against the spermathecal duct, which opens on the postero-ventral surface of the vagina in the mid-line. In this way the chances of fertilization would be improved. It seems possible that the vaginal tongue of *Nasonia vitripennis* functions in a similar way. From Fig. 2 (*b*, *c*) it can be seen that, when an egg's concave face is either dorsally or laterally orientated in the vagina, the tongue could press the egg's micropyle against the opening of the spermathecal duct; when the meridian of the egg is ventral as in Fig. 2 (*a*), however, the tongue would force the micropyle away from the duct of the spermatheca so that the sperm could not enter the egg. Therefore, on average one-quarter of the eggs laid would not be fertilized and so produce males. This suggests that all the eggs from one of the ovarioles may not be fertilized, and it accounts for the observed sex ratio of 21 per cent males in populations which had free access to host puparia[1].

When mated females are deprived of host pupæ for four days prior to oviposition, the proportion of males in the offspring increases to 38·9 per cent[1]. It is known that if eggs are not laid within two days of their reaching maturity a process of resorption begins. Until now it has been suggested that in many Hymenoptera a partially resorbed egg produces a female because it is in the ovary longer and so stands a better chance of being fertilized[4]. On the present hypothesis, an alternative explanation would be that the tongue cannot push the partially resorbed egg's micropyle against the spermathecal duct because the egg has lost its shape and rigidity, Fig. 2 (*d*). The egg is therefore not fertilized and produces a haploid male.

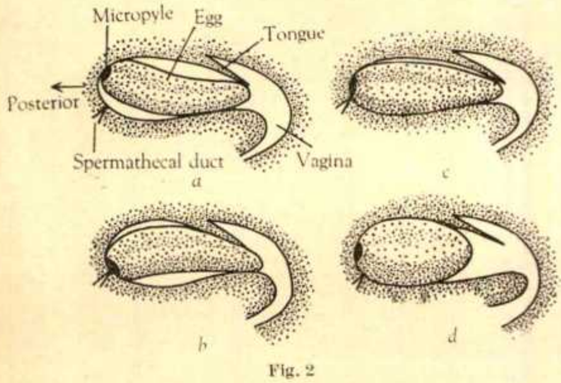

Fig. 2

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Occurrence of an Egg-String in *Enoplus communis* Bastian, 1865 (Nematoda : Enoplidae)

Adult members of the phylum Nematomorpha, residing in aquatic habitats, lay their eggs within long strings commonly referred to as 'egg-strings'. In the nematomorphs this egg-string is considered a secretory product of the antrum (a common chamber situated between the uteri and the cloaca and lined with a layer of glandular epithelium)[1]. Whereas the egg-laying habits of the nematomorphs are fairly well described, those of marine nemas are practically unknown. Hyman[1], in the Nematoda section of her invertebrate compilation, states that some marine nemas lay their eggs in an adhesive mass. Recently, the opportunity to elaborate on this observation was presented while I was studying marine nemas at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts.

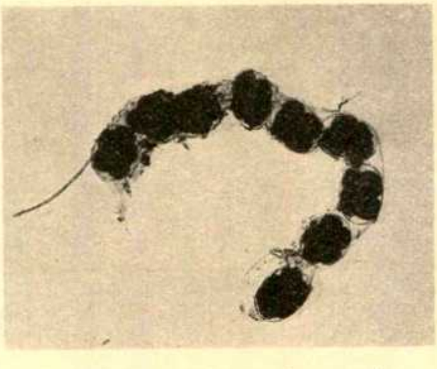

Fig. 1. 'Egg-string' of *Enoplus communis*

Live specimens of *Enoplus communis* Bastian, 1865, taken from the holdfasts of unidentified marine algae collected from Penzance Point at Woods Hole, were placed in a watch-glass in sea-water and left for 10–12 hr. Low-power examination of the specimens then revealed the presence of a line of 22 eggs. Higher magnification showed that each of the 22 eggs was contained within a continuous, transparent sheath (Fig. 1). In this respect the egg-containing sheath (egg-string) of *E. communis* appears to be analogous to that of the Nematomorpha. This sheath was attached to the bottom of the watch-glass, was adhesive, and contracted when probed with a dissecting needle. Presumably, the sheath anchors the eggs in habitats where they might otherwise be carried away or washed ashore by tidal action or else be subject to predation if deposited freely.

The sheath was at first thought to have arisen from vaginal (or vulvar) glands. However, on examination of *E. communis* females *in toto* and in cross-section, glandular structures were not observed. Histological studies are being conducted in an attempt to determine whether glandular tissue, possibly epithelium analogous to that of the antrum of the Nematomorpha, is associated with the distal portions of the reproductive tract.

It was also noticed that, though eggs within adult females were unsegmented, those in the egg-string were segmented and at the 6- to 8-celled stage of development.

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ANATOMY

Cholinergic Nervous Pathways in the Forebrain

In a previous communication[1] we described the use of a modified Koelle technique to produce selective staining of certain nuclei and tracts in the rat hindbrain. We have applied recently a similar method to the forebrain of normal animals and after transcortical lesions performed with an ophthalmic knife or cautery. The brains were removed after perfusion with 10 per cent formol saline, and 5–6 mm. transverse slices were afterwards fixed in the same fluid in the cold. To facilitate cutting, the tissue after fixation was soaked in 20 per cent alcohol, or embedded in an alginate gel according to a technique to be described elsewhere. The various media used for incubation contained either acetylthiocholine or butyrylthiocholine as substrates, with or without the addition of 10^{-4} M ethopropazine as a pseudocholinesterase inhibitor.

In sections of normal brains incubated with acetylthiocholine substrate, the forebrain tracts which stained selectively included the ventral supra-optic decussation, the central core of the fasciculus retroflexus, the cingulum, and the most lateral and most medial elements of the stria terminalis. In addition, stained fibres were interspersed in considerable numbers among the unstained fibres of the efferent projection pathways of the hippocampus: that is, in the alveus and in the dorsal fornix and fimbria behind the ventral hippocampal commissure. Caudally, most of the stained fibres of the dorsal fornix pierce the dorsal hippocampal commissure and so connect with the alveus, although some may connect with the cingulum via the corpus callosum. In the fimbria the stained fibres appear closely packed along its ventrolateral border (Fig. 1*a*). No fibres were seen in the descending columns of the fornix, and the crossed fibres of the ventral hippocampal commissure were likewise unstained. Below this commissure heavy staining due to true cholinesterase was rather unexpectedly found in the subfornical organ or intercolumnar tubercle (Fig. 1*b*), especially around its periphery, where a fine nervous plexus can be detected in sections stained by the Bodian silver method.

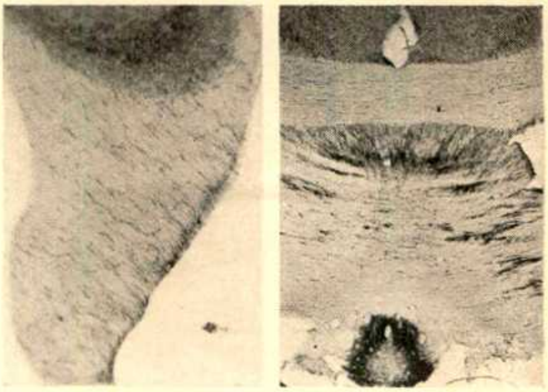
(*a*) (*b*)

Fig. 1. *a*, Stained fibres of fimbria. Blood vessels also present, unstained in sections incubated with pseudocholinesterase inhibitor. *b*, Stained fibres of cingulum above corpus callosum, and of dorsal fornix between corpus callosum and ventral hippocampal commissure. Stained cells belonging to nucleus of ventral hippocampal commissure. Subfornical organ heavily stained below commissure

A high concentration of true cholinesterase within the axon, such as has been demonstrated in all the tracts mentioned above, is characteristic of cholinergic neurons[2]. Having observed in the hindbrain that the cell bodies of such neurons are themselves rich in enzyme and stain heavily by the Koelle method[3], we have attempted to trace some of these presumably cholinergic pathways of the forebrain to their nuclei of origin. The lateral fibres of the stria terminalis appear to run caudally from cells of the bed nucleus of that tract to the unstained area occupied by the central amygdaloid nucleus. The medial fibres associated with the stria terminalis, on the other hand, have no connexion with either the lateral amygdaloid nucleus or the nucleus of the lateral olfactory tract, which are the two stained nuclei of the amygdala, but appear to run forward from the ventral nucleus of the lateral geniculate body, and curve downwards and backwards in front of the reticular nucleus of the thalamus. Sagittal sections show some fibres of the cingulum arising from small, deeply stained cells of the nucleus of the diagonal band; others are probably derived from similar cells of the medial septal nucleus. These two nuclei are also a likely source for the stained fibres of the dorsal fornix and fimbria, although there may be some contribution from the interstitial nucleus of the ventral hippocampal commissure. The cells of this nucleus stain intensely (Fig. 1*b*), but are larger than those of the medial septal and diagonal band nuclei, and are unusual in containing some pseudocholinesterase (found intraneuronally elsewhere in the forebrain only in the anterior nuclei of the thalamus). Our observations suggest, therefore, that the cholinergic neurons of the cingulum, dorsal fornix and fimbria belong to the afferent hippocampal system described by several workers, for example, Daitz and Powell[4] and Cragg and Hamlyn[5,6].

We have tried to confirm experimentally the polarity of the stained fibres related to the hippocampus in rats which have been subjected to lesions involving the tracts and allowed to survive four days. In these animals fibres caudal to the lesion fail to stain, whereas those immediately rostral to it dilate, stain more intensely than the equivalent fibres on the unoperated side, and become characteristically beaded[7]. We believe that this constant finding is due to enzyme accumulating on the cell body side of the axon adjacent to the cut and disappearing on the other side, as was found to occur in peripheral nerve by Sawyer[8], and indicates that the cholinergic fibres of the cingulum, dorsal fornix and fimbria are directed caudally. We hope to extend this method to investigate the polarity of other cholinergic tracts of the forebrain.

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Effect of Limb Ablation on Neurones in *Xenopus* Larvæ

In the spinal cord of larval Anura, the motor cells which innervate the muscles of the hind limbs form ventral horns distinct both in position and time of appearance from the series of primary motor cells whose axons run to the trunk muscles. In the tadpole of *Xenopus laevis*, there are about 4,000 ventral horn cells at the time of their differentiation from the mantle layer of the cord, a number which has fallen to 1,200 by metamorphosis some eight weeks later[1]. 'Histogenic cell degeneration'[2] accompanies the differentiation of these neurones, but on a scale much beyond that required merely to reduce their number to the adult-level. It seems that, for every ventral horn cell that persists, some eight or nine undergo cell death during development. Differentiation of the ventral horn is thus accompanied by a continuous turnover of the constituent neuroblasts.

If the larval hind limb is amputated at the thigh, a sequence of cellular changes is initiated within both the cord and the dorsal root ganglia. From the second to the third day after amputation, the number of cell degenerations within the ventral horn on the operated side is increased, and the total number of cells thereby temporarily diminished. While the degeneration which normally accompanies differentiation of the ventral horn can be temporarily abolished by X-rays, the additional cell deaths consequent on limb amputation are unaffected by radiation.

Fresh neuroblasts from the mantle layer, however, enter the ventral horn, and the deficit in cell number is made good. This movement of cells continues beyond the level required to restore the original total, and from the fifth to the fifteenth day after amputation the number of ventral horn cells on the operated side is greater than that on the other. Finally, a second phase of cell degeneration reduces the ventral horn to a third of its normal total of cells. This final destruction of neurones affects particularly the posterior two-thirds of the ventral horn, which is thus shown to be principally concerned with the innervation of distal limb segments. The leg only rarely regenerates when the operation is performed at late larval stages.

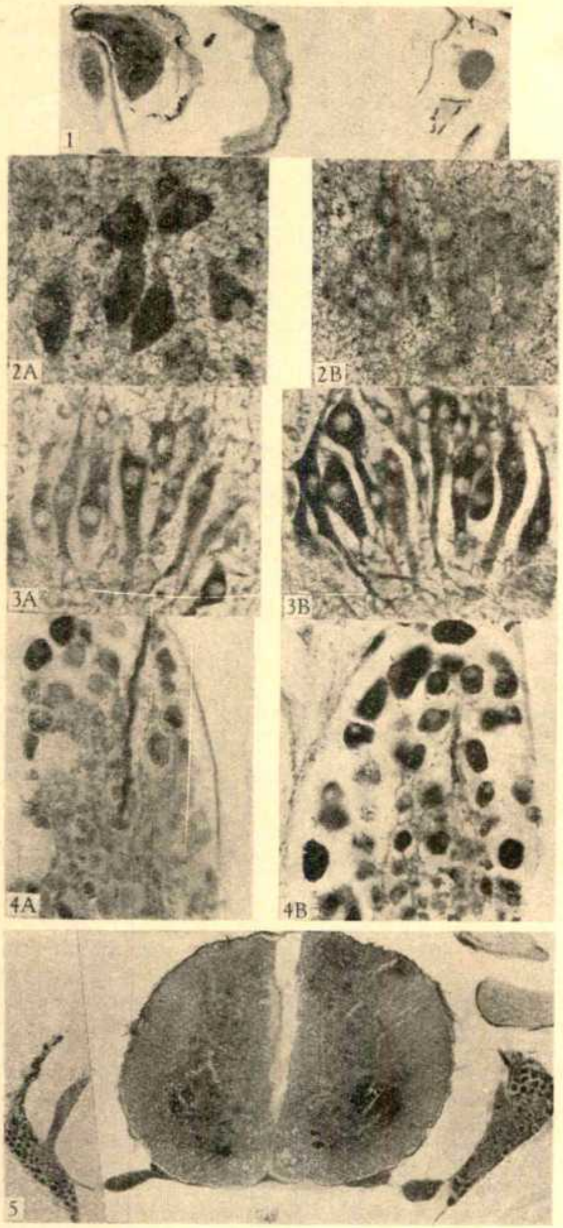
While these fluctuations in cell number are in progress, various changes are occurring in the ventral horn cells the axons of which have been severed by the operation. Attention was concentrated on changes in the Nissl substance and in the cholinesterase activity. Changes in Nissl substance were studied by ultra-violet absorption and controlled staining with basic dyes. Changes in the enzyme activity were studied by a special modification[3] of the thiocholine technique applied to small but intact specimens which were afterwards embedded in paraffin and sectioned. The sections were counterstained with buffered cresyl violet so that both Nissl substance and enzyme activity could be studied in the same neurone. In some of these preparations 10^{-4} M ethopropazine, a selective inhibitor of pseudocholinesterase, was used to confirm that only the distribution of true cholinesterase was being studied.

From the third to the fifth day, the ventral horn cells on the operated side show the typical reactions which were first described by Nissl in 1892 as 'chromatolysis', and have since been confirmed by numerous observers. The Nissl substance largely disappears, the cell cytoplasm becomes swollen, the nucleus moves towards the cell surface on one side, and the nuclear membrane becomes uneven in outline. By the eighth day, most neurones are recovering from this condition. The cytoplasm regains its normal dimensions, and, as the Nissl substance is re-formed, the chromosomal network within the nucleus becomes unusually distinct. In some of the largest cells this phase is succeeded by one in which cytoplasmic basiphilic material becomes densely piled round the nuclear membrane. This dense zone extends on opposite sides of the nucleus towards each pole of the cell. After the final phase of cell degeneration within the ventral horn, cells of this type are no longer to be found.

During the initial stages of chromatolysis the enzyme activity of the ventral horn cells on the operated side is at least as great as that on the control side and is often significantly greater (Figs. 2*A* and 2*B*). This enhanced enzyme activity is no longer present a week after ablation, and from then onwards an increasing proportion of cells on the operated side show only weak activity (Figs. 3*A* and 3*B*). Of such cells, although some are new immigrants which have not yet acquired the enzyme in any concentration, others are recovering from chromatolysis, and may show a dense perinuclear ring of basiphilic material. The weakly reacting neurones disappear from the ventral horn in the final wave of degeneration, after which the remaining cells, though of different sizes, are all strongly reacting (Fig. 5).

The most mature cells within the dorsal root ganglia of later larvæ show a weak but definite reaction for cholinesterase, the distribution of which can be studied by prolonging the incubation time in the thiocholine technique, and here, too, a sequence of changes is evident in those ganglia which supplied an ablated limb. Thus, after a week many of the cells no longer stain (Figs. 4*A* and 4*B*); during the third week many cells degenerate, leaving a ganglion which is much smaller than on the control, unoperated side but which contains about the same proportion of stained cells (Fig. 5).

Marked changes also occur peripherally. In the nerve trunk supplying a normal hind limb a proportion of the fibres stain for true cholinesterase. Following limb ablation this staining becomes much more intense, and individual fibres become noticeably distended (Fig. 1). Accumulation of enzyme in the severed axons is detectable near the cut ends within a few hours, and during the next few days it gradually increases in extent backwards towards the spinal cord at a rate of about half a millimetre per day. Later, this staining becomes less intense and eventually the fibres degenerate. Similar changes have been observed in the rat forebrain following lesions which interrupt cholinesterase-positive tracts to the hippocampus[4].



Figs. 1–5. All figures are from transverse sections prepared by the modified thiocholine technique through late larvæ of *Xenopus laevis* at various times after amputation of one hind limb. In each figure the operated side is shown on the left

Fig. 1. Through level of the sciatic nerves; 3 days after amputation. The nerve on the operated side is swollen and intensely stained

Fig. 2. Through level of the ventral horn; 4 days after amputation. The chromatolytic neurones, with displaced nuclei (*A*), are more intensely stained than the normal ones on the unoperated side (*B*)

Fig. 3. Through level of the ventral horn; 7 days after amputation. The post-chromatolytic neurones (*A*) are now less intensely stained than the normal ones (*B*)

Fig. 4. Through dorsal root ganglia supplying the hind limbs; 8 days after amputation. The proportion of strongly stained cells is much lower on the operated side (*A*) than on the normal side (*B*)

Fig. 5. Through dorsal root ganglia and cord; 18 days after amputation. There are now fewer cells in both the dorsal root ganglia and the ventral horn on the operated side

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VIROLOGY

Determination of the Concentration of Myxoviruses and of Some Specific Antisera, based on Neuraminidase Activity

Of the different properties of the viruses of the myxogroup, for example, infectivity, antigenic properties, hæmagglutination and enzymic activity, only hæmagglutination has so far provided moderately rapid and accurate titration methods for the determination of myxovirus concentrations. A disadvantage of these methods is the necessary use of red blood cells, which are difficult to standardize.

We have devised a method for determination of myxovirus concentrations, based on the enzymic activity of the virus particles. The action of the virus enzyme—its ability to destroy the hæmagglutination-inhibitory capacity of some mucoproteins or to elute the virus from red blood cells—always leads to the splitting off of a sialic acid, mostly N-acetylneuraminic acid[1]. This neuraminidase activity can be measured by determining the amount of free sialic acid released by the enzyme when the virus is incubated at 37° C. with a suitable substrate. As substrates we use the mucoprotein prepared by alcohol precipitation from human urine[2], or the compound of low molecular weight N-acetylneuraminyl-lactose isolated from cow

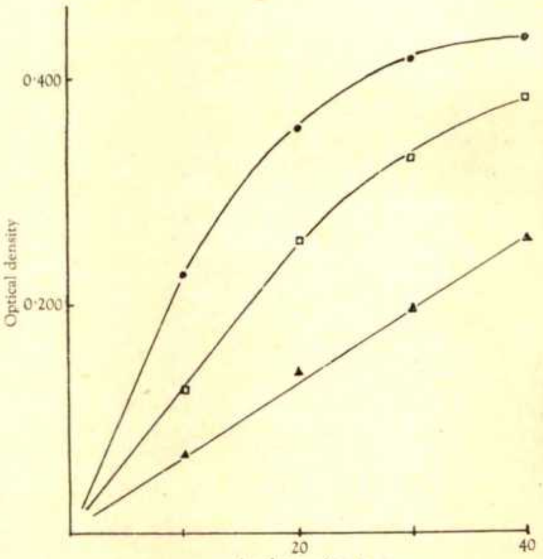

Fig. 1. Cleavage of N-acetylneuraminyl-lactose (100 μgm./ml.) by a sample of influenza virus neuraminidase (strain A_2-Singapore 1957) in several dilutions (▲, × 64; □, × 32; ●, × 16), as a function of the incubation time

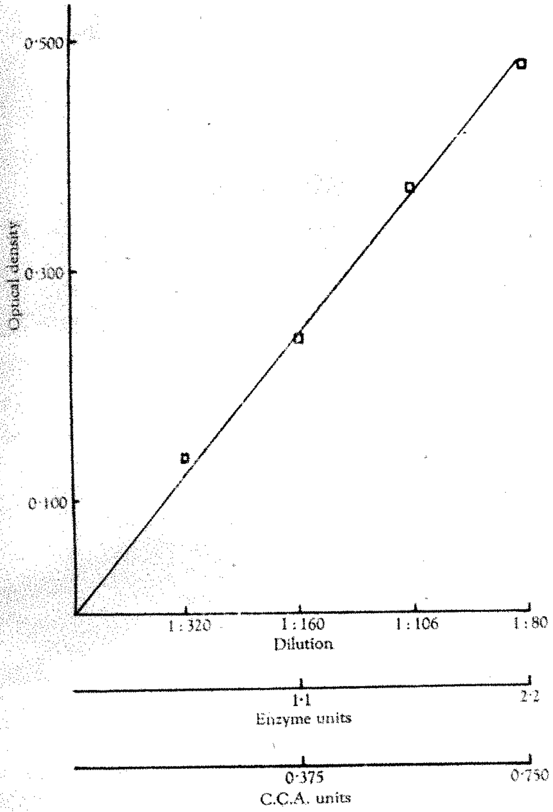

Fig. 2. Determination of neuraminidase (virus)-concentration, strain A_2-Singapore 1957 by cleavage of N-acetylneuraminyl-lactose. Relation between dilution factor and N-acetylneuraminic acid released (optical density) after incubation for 30 min. The virus dilutions on the horizontal axis are compared both with the amount of enzyme units in the sample (one unit representing the amount of enzyme that releases 1 μmole of N-acetylneuraminic acid in 1 min. under the pH and temperature conditions used) and with the amount of hæmagglutinin (chicken red cell agglutination units (ref. 6))

colostrum by a procedure rather similar to that used for N-acetyl-O-acetylneuraminyl-lactose[3]. The free N-acetylneuramic acid is estimated by the $NaIO_4$-thiobarbituric assay of Warren[4], which assay is negative for the N-acetylneuraminic acid, ketosidically linked in the substrate. We use the Warren method with some modifications such as a correction for slight cloudiness by subtracting the optical density at 600 mμ or by mixing the organic phase with 2·5 per cent v/v acetone to avoid any cloudiness. Cuvettes with a 4-cm. light path were used.

In Fig. 1 the action of a myxovirus enzyme is shown. During the incubation of the substrate (here N-acetylneuraminyl-lactose) and the enzyme—influenza virus A_2 1 (1957 Singapore)—at 37° C., samples are taken at various intervals for the determination of the N-acetylneuraminic acid release. By the addition to the samples of the strongly acid $NaIO_4$-H_3PO_4 reagent the enzyme reaction is stopped promptly. It may be seen that when the virus and substrate concentrations are rightly chosen, the amount of N-acetylneuraminic acid released is directly proportional to the incubation time. With this method it can further be shown that myxovirus neuraminidases follow Michaelis–Menten kinetics[5].

When a series of virus preparations of suitable dilution are incubated with the substrate for a standard time, the relationship between the N-acetylneuraminic acid released and the virus dilution factor is found to be linear (Fig. 2). It is thus possible to express the virus concentration in enzyme units; furthermore, the strength of an unknown virus suspension can be determined by measuring the enzymic activity of several dilutions of this suspension and comparing it with that of a standard. When the determination is performed with only one virus dilution, in the range where the effect varies linearly with the concentration, the accuracy is equal to that of the chicken red cell agglutination method[6].

We applied this method to the influenza virus strains: *A* PR_8(1934 U.S.A.), A_1FM_1(1947 U.S.A.), A_1 5(1953 Nederland), A_2 1(1957 Singapore), A_2 305(1957 Japan), *B* Lee(1940 U.S.A.), *B* Bon(1942 Australia), *B* 33(1958 Johannesburg), to *A* Swine influenza Swine 15(1930 U.S.A.) and to Newcastle disease, strain Hitchner *B*1. Determinations can only be carried out, however, with more or less purified virus suspensions, since crude allantoic fluid was found to contain inhibitory substances. The concentration of receptor-destroying enzyme in filtrates of *Vibrio cholerae* can be determined in the same way. It is interesting to note that the quotient neuraminidase units/chicken red-cell agglutination units varies strongly for the different virus types, being highest for the two *A*2 strains.

Myxovirus antibodies can be demonstrated by their inhibition of viral hæmagglutination. It seemed interesting to know if the viral enzymic activity is also blocked by antiserum. This proved to be true for the antibodies against two Asian influenza strains A_2 1(1957 Singapore) and A_2 305(1957 Japan), but not for some other strains, for example, A_1 5(1953 Nederland) and *B* Bon(1942 Australia). The concentration of the antibodies against the two Asian strains can be determined by measuring the degree of inhibition of enzymic activity (Fig. 3). In these antibody determinations we use monovalent vaccines, prepared with β-propiolactone, which retain their enzymic activity.

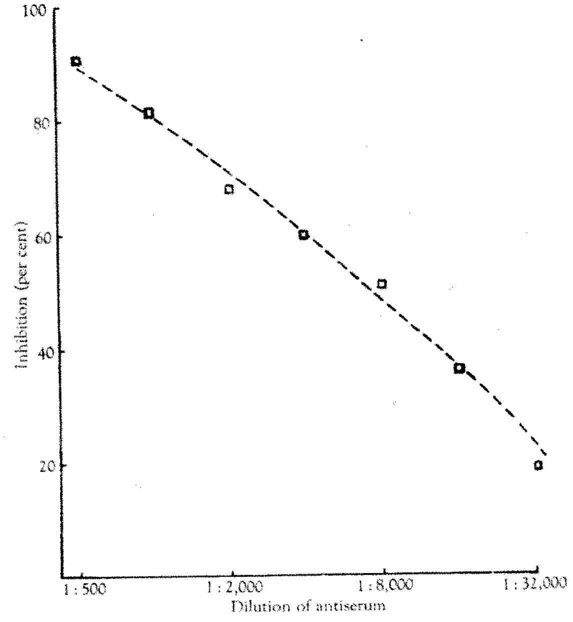

Fig. 3. Inhibition of neuraminidase activity of A_2-Singapore 1957 by homologous rabbit antiserum

This, and further work, will be reported in detail elsewhere. We acknowledge the technical assistance of Miss A. J. Schirmer.

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[1] Gottschalk, A., *Advances in Enzymol.*, **20**, 135 (1958). Zilliken, F., and Whitehouse, M. W., *Advances in Carbohydrate Chem.*, **13**, 237 (1958).

[2] Tamm, I., and Horsfall, jun., F. L., *Proc. Soc. Exp. Biol. Med.*, **74**, 108 (1950).

[3] Kuhn, R., and Brossmer, R., *Chem. Ber.*, **89**, 2013 (1956).

[4] Warren, L., *J. Biol. Chem.*, **234**, 1971 (1959).

[5] Walop, J. N., Boschman, Th. A. C., and Jacobs, J., *Biochim. Biophys. Acta*, **44**, 185 (1960).

[6] Miller, G. L., and Stanley, W. M., *J. Exp. Med.*, **79**, 185 (1944).

MISCELLANY

Sampling of Sub-micron Particles for Electron Microscopy

IN recent work on the attachment of radioactive particles to Aitken nuclei, we have been concerned with the sampling and subsequent examination by electron microscopy of Aitken nuclei in the size-range $0 \cdot 01$–$0 \cdot 1\mu$ diameter. A thermal precipitator was used for sampling, and the nuclei were at first collected on an electron microscope grid covered by a carbon film, instead of on the usual glass coverslip. Doubts arose as to whether the sample was a uniform one or whether there was preferential deposition over the copper bars of the grid by reason of the increased thermal gradient in the neighbourhood of the bars or by sagging of the carbon film between them. This effect has already been reported by Cartwright[1], who, to obtain uniform samples, used the thermal precipitator with the usual coverslips coated with a 'Formvar' film. After collection of the sample the film was scratched into sections of suitable size, floated off the coverslip in distilled water and placed on a grid for subsequent examination by electron microscopy. Cartwright observed preferential deposition on the grid bars of dust particles up to a few microns in diameter by means of an optical microscope, but in the present work the Aitken nuclei were too small for optical microscopy, and nuclei deposited over the grid bars could not be seen with the electron microscope.

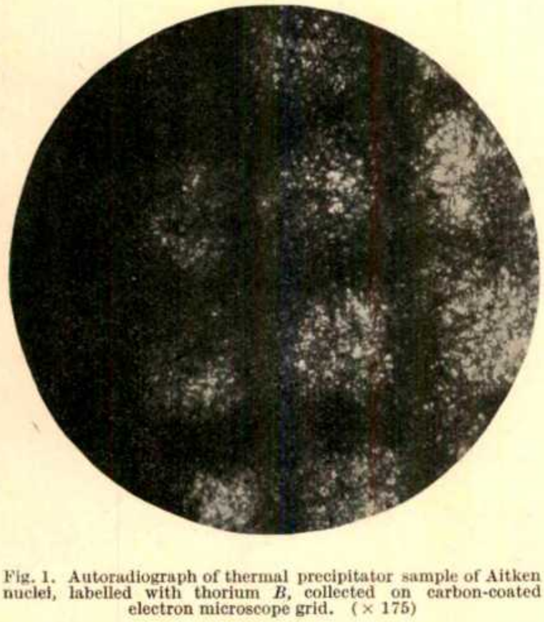

Fig. 1. Autoradiograph of thermal precipitator sample of Aitken nuclei, labelled with thorium *B*, collected on carbon-coated electron microscope grid. (× 175)

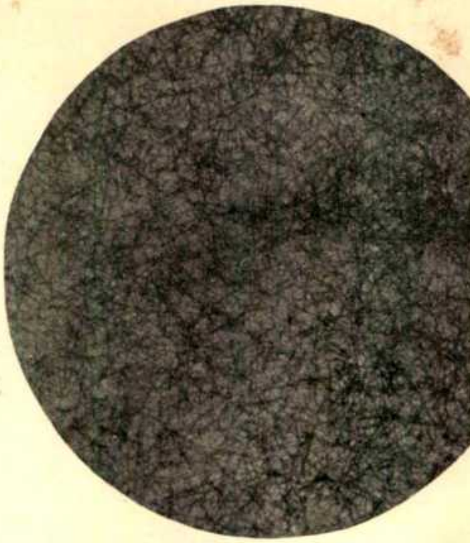

Fig. 2. Autoradiograph of simultaneous sample collected on carbon-coated coverslip. (× 175)

The technique finally used to test the uniformity of the sample was to mark the Aitken nuclei with atoms of thorium *B* and then to autoradiograph the samples. Areas where Aitken nuclei were concentrated could then be distinguished by the local concentration of alpha tracks. The autoradiographs shown are from the same thermal precipitator sample taken with an electron microscope grid (Fig. 1) and a coverslip (Fig. 2), both coated with an evaporated film of carbon, on opposite sides of the heated wire. It is apparent that the deposit on the coated coverslip is very much more uniform than that on the grid. Evaporated carbon films were used in preference to 'Formvar' because of ease of production and increased strength in the electron beam.

The method of marking the nuclei with thorium *B* was to pass filtered air at the rate of 200 cm.^{3}/min. through a solution of 5 mc. of radiothorium (thorium-228) in 40 ml. of $0 \cdot 05$ normal nitric acid. The thoron carried over was then passed into the vessel containing the nuclei to be marked. The short-lived thoron decayed in the vessel to give the thorium *B* atoms which attached themselves to the nuclei present.

Because of its low sampling-rate, the thermal precipitator is not a very convenient instrument in the present application, and we hope to develop a method of sampling on to carbon-coated coverslips by electrostatic precipitation.

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Harwell.

[1] Cartwright, J., *Brit. J. App. Phys.*, Supp. No. 3 (1954).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, January 30

University of London (in the Assembly Hall, Institute of Education, Malet Street, London, W.C.1), at 5 15 p.m.—Dr. P. F. R. Venables: "The Problems of Isolation in Technical Education".*

University of London (in the Anatomy Theatre, University College, Gower Street, London, W C 1), at 5.30 p.m.—Prof. J. P. Den Hartog (Massachusetts Institute of Technology) "Topics in Mechanical Vibrations. 1: Torsional Vibrations in Engine Systems caused by Gear Inaccuracies".* (Further lectures on February 1 and 2.)

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p m.—Dr. James Taylor "The Springs of Progress". (Second of three Cantor Lectures on "The Modern Chemical Industry in Great Britain")

Tuesday, January 31

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p m —Dr. W. A. Smeaton: "Science and Politics during the French Revolution".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 30 p.m.—Dr. H. E. M. Kay "Transplantation of Hæmopoietic Cells".* (Sixth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. L. S. Yoxall: "Instrumentation—Past, Present and Future".

Wednesday, February 1

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. F. G. J. Hayhoe: *"Cytology of Leukæmic Cells"*.*

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5 p.m.—Dr D. J. Bartholomew: "A Test of Homogeneity of Means under Restrictive Alternatives".

Royal Society of Medicine, History of Medicine Section (at 1 Wimpole Street, London, W.1), at 5 15 p.m.—Mr. J. T. Rowling: "Pathological Changes in Mummies"; Dr. P. A. Zorab: "The Historical and Prehistorical Background to Ankylosing Spondylitis"; Mrs. Glemser: "The Anthropological Applications of Blood Grouping".

British Institution of Radio Engineers, Radar Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. E. N. Davies "A Fast Electronically Scanned Receiving System".

Institute of Information Scientists (at the Swedenborg Society Hall, 20 Bloomsbury Way, *London*, W.C.1), at 6.15 *p.m.*—Mr. F. W. Cousins: "Language at the Interface of Technology and the Law".

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. J. C. Pinder: "The Economics of Agricultural Chemicals and Fertilizers".

Society for Analytical Chemistry, Physical Methods Group (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "X-Ray Fluorescence". Mr. K. M. Bills: "X-Ray Fluorescence Spectroscopy"; Dr. F. Brown: "Some Analytical Applications of X-Ray Fluorescence Spectrometry".

Thursday, February 2

Institute of Physics and the Physical Society (in the Physics Department of Imperial College, Prince Consort Road, South Kensington, London, S W.7), at 2.45 p.m.—Dr. J H. Sanders: "The Optical Maser"; Dr. W. R. Hindmarsh: "Collision Broadening and Shift in Atomic Spectra".

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m —Prof. W. D. M. Paton, F.R.S.: "A Theory of Drug Action Based on the Rate of Drug-receptor Combination"; Mr. B. B. Boycott, Mr. E. G Gray and Mr. R. W. Guillery: "Synaptic Structure and its Alteration with Environmental Temperature: a Light and Electron Microscope Study of the Central Nervous System of Lizards".

Institute of Refrigeration (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. Carl Munters and Mr. Lennart Lindqvist: "A New Conception in Cooling Tower Design".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. J. Blundell, Mr. A. E. Garside, Mr. R. G. Hibberd and Mr. I. Williams: "Silicon Power Rectifiers".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C 1), at 5.30 p.m —Dr C. E. Ford · "The Inter-relationship of Nucleus and Cytoplasm".* (Seventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (in the New Physics Building, Imperial College of Science and Technology, London, S.W.7), at 5.30 p m.—Prof. A. B. Pippard, F.R S.: "The *Electronic Structure of Metals*".* (First of three lectures. Further lectures on February 9 and 16.)

Institute of Metals, London Local Section (at 17 Belgrave Square, London, S.W.1), at 6 30 p.m.—Mr. P. G. Forrester: "The Development of Some New Bearing Materials".

University of London (at Wye College, Wye, near Ashford, Kent), at 8.15 p.m.—Prof. R. Brown: "Morphogenesis in Plants".*

Friday, February 3

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W.C.2), at 6 p.m.—*Discussion on* "Recent Technical *Developments* in Eye Movement Recording" opened by Dr. P. A. Merton and Dr. C. Rashbass.

Society of Chemical Industry, Road and Building Materials Group (joint meeting with the Manchester Section, in the Robinson Theatre, The University, Manchester), at 6 p.m.—Mr. D. H. Matthews: "Surface Active Agents in Bituminous Road Materials".

Royal Institute of Chemistry, London Section (joint meeting with the Brighton Technical College Chemical Society, at Brighton Technical College, Brighton), at 7.30 p.m.—Sir H. W Melville, F.R.S.: "Research and Development in D.S.I.R. Stations".

Saturday, February 4

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E 23), at 3.30 p.m.—Dr. F. S. Wallis: "Prehistoric Stone Factories".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Biochemist (Principal grade), to take charge of the Biochemical Department of the Group Laboratory, Lakin Road, Warwick—The Group Secretary, South Warwickshire Hospital Group, 50 Holly Walk, Leamington Spa (February 2).

Assistant Lecturer or Lecturer in the Department of Pure Mathematics—The Registrar, The University, Liverpool (February 3).

Lecturer or Assistant Lecturer in Inorganic Chemistry—*The Clerk to the Governors*, Chelsea *College of Science* and Technology, Manresa Road, London, S.W.3 (February 8).

Lecturer in Agriculture (Animal Production); and a Departmental Assistant in *Animal Physiology*, at *the School of* Agriculture, Sutton Bonington—The Registrar, The University, Nottingham (February 4).

Lecturer (with graduate qualifications and wide experience of the paper-making industry) in Paper Technology—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent (February 4).

Research Assistant (with a degree in mathematics or equivalent qualifications, and preferably previous experience of electronic computers) in the Computing Machine Laboratory, to assist in the operation of a computing service on the Mercury and Atlas electronic computers—The Registrar, The University, Manchester 13 (February 4).

Senior or Junior Research Assistant, for the M.R.C. Research *Group in Enzymology in the Department* of Chemical Pathology—The Secretary, St. Mary's Hospital Medical School, Paddington, London, W.2 (February 4).

Lecturer (male) in *Education*—The *Principal, Borough Road* College, Isleworth, Middlesex (February 6).

Assistant Lecturer in Philosophy—The Registrar, The University, Leicester (February 10).

Lecturer in Physics; and a Lecturer in Physiological Psychology—The Registrar, The University, Leicester (February 10).

Lecturer in Pure Mathematics; a Lecturer in Applied Mathematics; an Assistant Lecturer in Pure Mathematics; and an Assistant Lecturer in Applied Mathematics—The Registrar, The University, Leicester (February 10).

Lecturer (with special interest in genetics) in Botany—The Registrar, The University, Leicester (February 10).

Lecturer or Assistant Lecturer in Statistics in the Department of Pure Mathematics—The Registrar, The University, *Edgbaston, Birmingham* 15 (February 15).

Reader in Applied Mathematics—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (February 15).

Assistant Lecturer (with a good honours degree in either botany or agricultural botany, and a special interest in mycology or plant pathology) in the Department of *Agricultural Botany*—The Registrar, University College of Wales, Aberystwyth (February 18).

Reader in Pharmacology; a Lecturer in Sociology; a Lecturer in Organic Chemistry; a Lecturer in Food Science, a Lecturer in Agriculture (Animal Production); Lecturers and Assistant Lecturers in Civil Engineering and Electrical Engineering; and Assistant Lecturers in Applied Mathematics and Botany—The Registrar, The University, Nottingham (February 18).

Lecturer in Aeronautical Engineering—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (February 20).

Reader in Applied Mathematics at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 *(February 24)*.

Fellow and Lecturer (mathematician with interests in the field of theoretical physics) in Mathematics—The Rector, Exeter College, *Oxford (February* 25).

Lecturer or Assistant Lecturer in Applied Mathematics—The Secretary and Registrar, University College of North Wales, Bangor, North Wales (February 27).

Assistant Lecturer or Lecturer (with qualifications in any branch of pure mathematics and special interests in numerical analysis or mathematical statistics) in the Department of Mathematics; and an Assistant Lecturer (with special qualifications in inorganic chemistry or in physical chemistry) in the Department of Chemistry—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (February 28).

CHAIR OF MATHEMATICS in the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

LECTURERS OR ASSISTANT LECTURERS (3) IN PHYSICS—The Registrar, The University, Hull (February 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, BIOCHEMISTRY or GEOLOGY—The Registrar, The University, Leicester (March 4).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOW IN CHEMISTRY AND PHYSICS—The Secretary, The University, Exeter (March 5).

ASSISTANT LECTURER IN CHEMISTRY; an ASSISTANT LECTURER IN GEOGRAPHY; and an ASSISTANT LECTURER IN PURE OR APPLIED MATHEMATICS at the University of Singapore, Malaya—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 31).

IMPERIAL CHEMICAL INDUSTRIES FELLOWS FOR RESEARCH IN PHYSICS, CHEMISTRY, COLLOID SCIENCE, BIOCHEMISTRY, ENGINEERING, METALLURGY, PHARMACOLOGY, CHEMOTHERAPY, or related subjects—The Secretary General of the Faculties, The University, The Old Schools, Cambridge (April 29).

DEAN OF THE FACULTY OF HOME SCIENCE, University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, April 30).

GEOLOGISTS (4), to teach in the fields of mineralogy, petrology, petrography, palæontology, stratigraphy, structural geology, economic geology and geophysics—Escola de Geologia, Universidade da Bahia, Salvador, Bahia, Brazil.

MASTER TO TEACH SCIENCE, CHIEFLY PHYSICS AND CHEMISTRY—The Headmaster, King's College, Taunton, Somerset.

RESEARCH ASSISTANT (with an honours degree and preferably some design experience), to investigate high strain fatigue of metals (work suitable for higher degree)—Prof. H. Ford, Imperial College of Science and Technology, London, S.W.7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

National Association for Mental Health. Annual Report, 1959–60. Pp. 55. (London: National Association for Mental Health, 1960.) [2710

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 701, Vol. 243 (27 October 1960): Coastal Interglacial Deposits of the English Channel. By R. G. West and B. W. Sparks. Pp. 95–133 + plate 16. (London: The Royal Society, 1960.) 14*s*. [2710

British Broadcasting Corporation. B.B.C. Engineering Monograph No. 32: A New Survey of the B.B.C. Experimental Colour Transmissions. By I. R. Atkins, A. R. Stanley and S. N. Watson. Pp. 31. (London: British Broadcasting Corporation, 1960.) 5*s*. [2710

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1024, Vol. 253 (27 October 1960): The Boltzmann Equation for a Bounded Medium. 1: General Theory. By S. Simons. Pp. 137–184. (London: Royal Society, 1960.) 15*s*. [3110

Ciba (A.R.L.), Limited. Technical Notes, No. 214 (October 1960): Radio-frequency Heating, Part 2 (conclusion). Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1960.) [3110

Hopkin and Williams, Ltd. Organic Chemical Reagents Monograph No. 41: 4 : 7-Diphenyl-1 : 10-Phenanthroline ("Bathophenanthroline"): Reagent for Iron. By E. J. Newman and G. Peters. Pp. 7. (Chadwell Heath: Hopkin and Williams, Ltd., 1960.) [3110

The International Association for the Exchange of Students for Technical Experience. Thirteenth Annual Report, 1960. Pp. 70. Report of I.A.E.S.T.E. in Great Britain for year ending September 1960. Pp. 12. (London: International Association for the Exchange of Students for Technical Experience, 1960.) [3110

Geomorphological Abstracts, No. 1 (June 1960). Pp. 23. Published four times a year in March, June, September and December. (London: K. M. Clayton, Department of Geography, London School of Economics and Political Science, 1960.) [3110

English Electric Valve Co., Ltd. E702 Storage Tubes. Pp. 28. (Chelmsford: English Electric Valve Co. Ltd., 1960.) [3110

The Registrar General's Statistical Review of England and Wales for the year 1958. Part 3: Commentary. Pp. xiv + 222. (London: H.M. Stationery Office, 1960.) 13*s*. net. [3110

Forestry Commission. Report on Forest Research for the year ended March, 1959. Pp. ix + 186 + 5 plates. 9*s*. 6*d*. net. Leaflet No. 2: *Adelges cooleyi*: an Insect Pest of Douglas Fir and Sitka Spruce. By Dr. Myles Crooke. Pp. 8. 9*d*. net. (London: H.M. Stationery Office, 1960.) [3110

Ministry of Agriculture, Fisheries and Food. Bulletin No. 115: Construction and Heating of Commercial Glasshouses. Pp. iv + 98 + 8 plates. (London: H.M. Stationery Office, 1960.) 6*s*. net. [3110

Scottish Marine Biological Association. Annual Report, 1959–60. Pp. 28. (Millport, Isle of Cumbrae: Scottish Marine Biological Association, 1960.) 4*s*. [3110

Department of Scientific and Industrial Research: Building Research Station. National Building Studies Research Paper No. 31: Studies on Bridge-Deck Systems. Part 2: Bending Moments in a Plate-and-Girder System. By C. C. Ritchie. Pp. iv + 67. (London: H.M. Stationery Office, 1960.) 4*s*. net. [711

Institution of Gas Engineers. Publication No. 578: The Choice of Subjects for Research and Development. By Sir Harold Hartley. Pp. 9. (London: Institution of Gas Engineers, 1960.) [711

British Universities Film Council. Catalogue of Films for Universities. Pp. 166 + xxiv. (Glasgow: British Universities Film Council, Royal College of Science and Technology, 1960.) [711

Building Research Station Digest. Second Series, No. 4: Repairing Brickwork. Pp. 4. (London: H.M. Stationery Office, 1960.) 4*d*. [711

Planning, Vol. 26, No. 446 (7 November, 1960): More Planning in Pakistan. Pp. 309–336. (London: Political and Economic Planning, 1960.) 3*s*. 6*d*. [711

Other Countries

Fisheries Research Board of Canada. Biological Station, St. Andrews' N.B. General Series Circular No. 33 (September, 1960): Outlook for the Georges Bank Scallop Fishery. Pp. 2. (St. Andrews, N.B.: Fisheries Research Board of Canada, 1960.) [2410

Towards the Dissolution of the Thermodynamic Paradoxes. By Sven Svantesson. (Supplement to "Foundations of Thermodynamics, with Antitheses and Disputation.") Pp. 4. (Stockholm: Almqvist and Wiksell/Gebers, 1960.) [2410

Food and Agriculture Organization of the United Nations, Rome. Animal Health Branch Monograph No. 1: Diseases of Free-Living Wild Animals. By Dr. A. McDiarmid. Pp. iii + 91. (Rome: Food and Agriculture Organization of the United Nations, 1960.) [2610

Indian Forest Bulletin No. 171 (3), (New Series): A List of Insect Pests of Forest Plants in India and the Adjacent Countries. Part 4: List of Insect Pests of Plant Genera 'C' (concluded) (*Clausena* to *Cytisus*). By R. N. Mathur and Balwant Singh. Pp. 45. Rs. 5.24nP.; 8*s*. 3*d*. Indian Forest Bulletin No. 171 (4), (New Series): A List of Insect Pests of Forest Plants in India and Adjacent Countries. Part 5: List of Insect Pests of Plant Genera 'D to F' (*Dactyloctenium* to *Funtumia*). By R. N. Mathur and Balwant Singh. Pp. 165. Rs. 10.; 16*s*. Indian Forest Leaflet No. 161 (Entomology), *Chrysocraspeda olearia* Guénee, a Defoliator of *Eugenia Jambolana* (Geometridae: Lepidoptera). By R. N. Mathur. Pp. 4 + 1 plate. Rs. 0.87nP.; 1*s*. 3*d*. Indian Forest Records (New Series). Silviculture. Vol. 10, No. 8: General Standard Volume Tables for *Casuarina equisetifolia* Forest. By S. K. Seth and S. N. Dabral. Pp. 227–237. Rs. 2.75nP.; 1*s*. 6*d*. (Delhi: Manager of Publications, 1959 and 1960.) [2710

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STRENGTHENING SCIENCE AND TECHNOLOGY

MORE than a year has elapsed since an eminent group met in Paris to discuss ways and means of strengthening science in the Western world. As scientists with executive ability, they had been selected from countries within the North Atlantic Treaty Organization. Motives for bringing together such a study group were prompted partly by the Cold War. However, "even if there were no Soviet menace . . . the demands of the populations of the Western countries for a higher standard of living, for relief from toil and drudgery, for increasing health of body and mind, can only be met by the advance of science and its applications in technology". A report was issued*, and the gist of it appears in a leading article of *Nature* (188, 880; 1960). During December 19–23, countries of the Middle East and North Africa sent representatives to a meeting in Cairo to confer on ways of invigorating science and technology in their respective lands. This was held under the hovering auspices of Unesco, and the conference is reported on p. 362 of this issue of *Nature*. It is of interest to examine critically some of the points which arose from these meetings. Although the participants in the two meetings came from countries which vary widely in the degree of technological development, many of the obstacles encountered are common to all.

The outcome of these deliberations is very cogent and deserves praise. In particular it is heartening to see a resolution from the Cairo meeting (No. 17): "That all efforts and results reached by science should be used solely to help humanity. . . ". It has never been denied that a vast amount of scientific effort is directed according to military considerations. That such research lends an inevitable impetus to the scientific quest for truth is not wholly valid. The effect is of second-order importance—almost trifling, in fact. Perhaps this resolution portends that scientists, realizing the implications, will begin to emerge from their research cartels, exercise a certain political awareness and give a resounding, concerted negation to what amounts to a greater and greater refinement of preparation for cataclysmic war.

To achieve scientific and technological prowess, education is necessary, so much has been said about instructing the would-be scientist. In the Paris meeting it was considered that the logical place to start moulding the scientist and technologist was in the secondary school. That the claims of science and technology were very often subordinated to those of traditional courses in the humanities and law was deplored. Scientific interest should be aroused in the pupil. Quite often this was inhibited by conservative proclivities of teachers devoted to "inert ideas". Pupils should be able to relate the substance of their courses to what science is achieving, hence curricula should be topical. Sir John Cockcroft believes, quite rightly, that to strengthen Britain's science and technology it is more important to do so in the universities than anywhere else at the present time. On January 3 at Nottingham he stated that Britain needs a major new technological university of at least five thousand students if it is to catch up with the U.S.S.R. and the United States. Although during 1958–59 British students of the arts outnumbered slightly those reading science and technology, it seemed likely that by the late 'sixties there would be about twice as many technologists as students of the arts; the absolute number of the latter is not likely to diminish. He also alluded to a point which arose in the Paris meeting, namely, the possible foundation of an Atlantic institute of science and technology which would be a combined effort from countries of the North Atlantic Treaty Organization.

These various points on education for scientists and technologists are well taken. But perhaps a greater stress should be placed on quality rather than quantity. More should not mean worse. Large technological institutes have their place, but, after all, the birth of the Berlioz orchestra has never displaced the æsthetic need for the string quartette. The dangers of egalitarianism and conformity in large institutions are immense. In his book, *Teacher in America*, Prof. Jacques Barzun of Columbia University, New York, has pointed out the sorry plight of education in the United States. He feels that education should be a hand-to-hand affair. He refers to the 'preceptorial' system used successfully at Princeton University. This is a small university relative to the massive institutes of technology, but its educational calibre, both in the sciences and in the humanities, is unsurpassed in the United States. The preceptorial system is similar in principle to the tutorial system of Oxford and Cambridge, and possibly a few other universities, whereby students receive instruction in small groups. Standards of all degrees should be kept high, otherwise they become meaningless. It is quite revealing to examine some theses toward research degrees—their tortuous manipulation of symbols, numerous data, wealth of bibliography, but sadly a paucity of ideas. One is tempted to say with Virgil:

> "Why not apply yourself to useful tasks,
> Plait wicker crates and baskets of soft reeds?"

The probing spirit should be respected for what it is; not make a passport to probe, appended to it, a *sine qua non*. Perhaps atmosphere is lacking, and this could be partly ascribed to the passion for the research 'project'. The meditative approach was used by great men of science in the past—"What interesting questions does Nature pose and what is the minimum amount of experimental equipment needed to answer them ?"—and it resulted in great works. It is well worth a visit to the Cavendish Laboratory to see with what little equipment so much was accomplished.

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* Increasing the Effectiveness of Western Science. Pp. iii+19. (Brussels: Fondation Universitaire, 1960.)

The criticism levelled at academic institutions which encourage the humane tradition in education is perhaps not entirely called for. Science should not displace the humanities but be an adjunct and partner. The scientific method is one way of approaching truth progressively—there are other ways, equally laudable. An educated man should have reasonable command over all these. How disquieting to envisage a growing host emerging from technological institutes, all thinking in the same way, all with similar utterance—in effect, a band of æsthetically impervious, socially unaware, but adept barbarians who will not deem any knowledge worthy unless it be formulated exactly. It is Anton Tchekov who tells Grigorovitch he had come to the conclusion that "an artist's instinct may sometimes be worth the brains of a scientist, that both have the same purpose, the same nature, and that perhaps in time, as their methods become perfect, they are destined to become one vast prodigious force which now it is difficult even to imagine".

That science syllabuses are kept up to date and the pupil's enthusiasm stimulated by emphasizing present scientific achievements is necessary. However, it is well to point out to the pupil what fundamental scientific thought means as opposed to the technological aspects. His imagination is captivated easily by the glamour of rockets and satellites. This to him is science *par excellence*. However, if he were to realize that it is the genius of Newton's laws plus a fuel of large enough specific impulse, developed with abundant funds, which enables 'rocketeers' to 'aim at the stars', it would sharpen his critical appreciation of scientific merit.

The dilemma faced by a research worker seeking specialized information in his field was discussed by the group in Paris and at the meeting in Cairo. The recent decline in adequate documentation was blamed on "the mushroom growth of scientific publications" and on poor methods of classifying publications. Unesco and allied organizations will attempt to improve the situation by unifying editorial practice, by instituting a single international indexing system, by requesting authors to submit abstracts with their research articles and by discouraging new publications. How avid scientists and engineers are to-day to get into print ; to place their morsel on the altar of fame ! Inundated by this spate of material of greater or lesser virtue, one cannot help but yearn for the modesty of the Darwins and Faradays of the past. To the solutions which Unesco will attempt, it seems appropriate to add a wily suggestion given by Mr. L. J. F. Brimble during an address to the Royal Society of Edinburgh on January 11, 1954. He felt that every scientist intending to publish research should be posed with the following questions : "(1) Are you sure you have said what you want to say ? (2) Have you said it in the minimum number of words ? (3) Is it worth saying at all ?"

EVOLUTION

Evolution After Darwin

Edited by Prof. Sol Tax. Vol. 1 : The Evolution of Life—Its Origin, History and Future. Pp. viii+629. Vol. 2 : The Evolution of Man—Man, Culture and Society. Pp. viii+473. (Chicago : University of Chicago Press ; London : Cambridge University Press, 1960.) 80*s*. net each volume.

Evolution

Its Science and Doctrine—Symposium presented to the Royal Society of Canada in 1959. Edited by Thomas W. M. Cameron. ("Studia Varia" Series, Vol. 4.) Pp. xi+242. (Toronto : University of Toronto Press, 1960. Published for the Royal Society of Canada.) 40*s*. net.

THE first of these books (Vols. 1 and 2) forms part of a series of three volumes of Darwin entennialc essays written by well-known scientists as the basis for discussions which took place between the writers during the Darwin centennial celebrations at the University of Chicago, 1959. The third volume, *Issues in Evolution*, is not included in this review. Altogether there are 42 essays and some of them are very long, up to 80 pages and with about 200 references. These essays are quality writings, perhaps designed more for experts than for general readers, who would require a large background knowledge to appreciate all of them.

About a year ago I heard a publisher publicly declare that evolution as a topic of concern was dead. I suppose this is true in terms of the old Darwinian controversies. It seems that there is now a more settled and intelligent view of the matter—the theory has been assimilated into the general intellectual background. The publisher's statement is certainly not true when we turn to biological literature, and the symposia presented by the books reviewed are evidence enough of the professional interest in evolution. There are still plenty of teaching and research biologists who would answer the question "Is evolutionary theory important ?" with "Yes, I suppose so". They accept it, but are not particularly stimulated by it. It is precisely to people like this that the present symposia should be of importance, if only because these essays range over such a very wide field. The essays will either provoke the 'bandwagon' biologist's anger or his acceptance, but they will not leave him unmoved.

These essays show unmistakably how intertwined all the sciences are. Biology is not just the study of the grosser aspects of plants and animals, but now takes in all empirical approaches to knowledge in its phenomenological aspects. This all springs from the uniqueness of man, from mind if you like, but, as some of the essays show, this knowledge stops there. A disheartening feature of the books as a whole is that no indication is given of a further step in the study of life, particularly in relation to man, where much of the cultural tradition of the older scholasticism, which rendered possible the development of the newer scientific scholasticism, is ignored. An essay by Teilhard de Chardin, if it had been possible, or by someone of similar cosmic outlook, would have completed the story of evolution as set out in these two volumes. An essay taking in man as a biological and as a spiritual creature would have set a truer balance between the essays. The lack of such a balance has destroyed the harmony of the whole plan, and this disharmony is emphasized in several of the essays in which a materialistic approach (as distinct from a necessary mechanistic approach) is maintained. Other essayists, such as C. H. Waddington, are not so dogmatic. He contends, reasonably enough, that the evolutionary theory does not critically answer the question of the acceptance or rejection of theism or atheism. It must be settled in some other way, and so he is not concerned with it. But if evolution is the process basic to the whole of the cosmos, and it is to this view that a wider view of evolution leads, then the question of theism or atheism is of the utmost importance, for either evolution leads to one or the other concept or the process of evolution lacks meaning for man and is relatively unimportant.

While all the writers justifiably emphasize the importance of the works of Charles Darwin and many of them seek explanations through natural selection, some writers tend to discuss the operation of selection in fields where there is no evidence of its operation. An interesting feature of the books as a whole is that the importance of selection is not now so over-emphasized, except perhaps by a few extreme selectionists, but the importance of a balance between the well-known factors of evolution is acknowledged.

In the essays on the evolution of man, L. S. B. Leakey gives an interesting account of the new genus *Zinjanthropus* and its importance. But in some of these essays it is clear that there is a general attempt to lessen the Cartesian split between body and mind and hence between man and lower animals. The method of doing this would seem to be unsound, for man is played down as it were, his really important attributes are glossed over in favour of finding him a place in a zoological classification, while at the same time the animal is played up until we are nearly led to think that there is scarcely any fundamental difference between man and animal. But, to a non-Cartesian who still holds to the spirit of the Thomists that the phrase *animal rationale* is meaningful, the evidence for the smallness of the gap between man and animal does not seem excessively strong. Although to some biologists the term 'man' may be a confounded nuisance, to other people it is far more than a colloquial word. I was beginning to think that perhaps the infra-human hominoids were more human than I myself, until I was brought back to earth by recalling a sentence of Humphrey J. T. Johnson that there is a wider difference "between a man and a gorilla than between a gorilla and a daisy. The one is as incapable as the other of creating civilization".

All these essays are interesting and, above all, most stimulating. They are full of sound information and points of view and cover most aspects of evolutionary theory and its implications, but not all. An important essay which is missing from this work is one which discusses the validity of the fundamental assumptions of all evolutionary theory, the value of the mathematical models, particularly in the genetical field and the tautology of selection. There are biologists, such as W. R. Thompson, who doubt some of these assumptions, such as that embodied in G. G. Simpson's twisted phrase of "the genetical relationships that are reconstructed as phylogeny". Indeed, Bernard Rensch has contributed a very valuable essay on the laws of evolution which would have better earned its *droits de cité* if it had been preceded by an account of the methodological bases of evolutionary theory. A critique of evolutionary theory has long been needed and the opportunity for its production was given by these centennial symposia, but, at least in these books, the opportunity was lost.

Evolution: Its Science and Doctrine is also the result of a symposium on evolution organized by the Royal Society of Canada to commemorate the Darwin centenary and held at the annual meeting of this Society in 1959. The book contains twenty essays, all by Canadians. These essays do not cover such a wide field as those in the Chicago symposia, and most of them are straightforward accounts of biological, geological, biochemical, sociological, philosophical and cosmological aspects of evolution. They are less for the expert than for the general trained reader. They are non-controversial except for one of them. This last essay gives a biochemical outlook on evolution which is so dogmatically materialistic and narrow in its interpretation as to lower the intellectual level of the book as a whole. It is, however, offset to some extent by an essay in French on philosophy and evolution. It is unfortunate that this symposium did not include an essay on the evolution of man. In general this is an interesting book containing much useful information and with a balanced outlook on evolutionary matters.

P. G. FOTHERGILL

A FARRAGO OF EARTH HISTORY

Biography of the Earth

Its Past, Present, and Future. By Prof. George Gamow. Revised edition. Pp. xiii+242+27 plates. (London: Macmillan and Co., Ltd., 1959.) 35*s*. net.

IN his presidential address at Cardiff to the British Association Sir George Thomson emphasized the obligation of "the popularizer to take anxious care lest in trying to simplify he make statements which are simply untrue". This book is a most unhappy example of not taking care. Prof. Gamow has a well-deserved reputation as a popularizer, and I have myself had so much entertainment and pleasure from some of his books that it was with real distress that I found in the first edition of this book (1941) so much that was misleading and irresponsible. One trivial example sent a wave of hilarity around the geological world: on p. 145 Gamow had written "the name 'Cambrian' indicates simply that the deposits of that period were first discovered and studied in Cambridge-shire (England)". In the 'revised' edition of 1959, p. 145 remains exactly the same, word for word, as before. On the same page, and the next, we still learn that "micaschists" are ordinary sandstones "metamorphized". Indeed, nearly all the pages are reproduced without change. The main alteration is in the treatment of the origin of the solar system.

Collision hypotheses are now abandoned in favour of Weizsäcker's modified version of the Kant–Laplace hypothesis. Two or three pages have been revised to include the possibility that life began with the natural synthesis of amino-acids and their eventual linking together as proteins. Piltdown man is disowned as a fake. The age of the Earth is increased from 2,000 m.y. to 5,000 m.y.; but no mention is made of the magnificent work of Claire Paterson and his co-workers; the credit all goes to the rubidium–strontium method, which we are told gives more reliable results than those based on the decay of uranium, and has "more than doubled the estimated age of various rocks". Despite this, however, it is still stated that 1,500 m.y. "definitely represents the upper possible limit" for the age of the oceans. "Before that time all the Earth's water must have existed solely in the form of atmospheric vapour."

Corresponding to these erroneous ideas, Gamow tells us that the first extensive period of sedimentation was followed by the Laurentian revolution (about 1,000 m.y. ago). Because these layers of sediment contain but little salt, we are asked to believe "that sea water must have been quite fresh at that time". Not a word about the older orogenic belts, going back to 3,000 m.y. and beyond. Not a word about granitization, one of the most important processes affecting the Earth's biography. Seismologists will be depressed to learn that according to Gamow "the outer layer of the crust consists of a layer of granite extending to a depth of from 50 to 100 kilometres. This granite layer rests on a layer of heavier volcanic material, known as basalt, which is probably several thousand kilometres deep". Nowhere do we find a word about the Moho discontinuity or the astonishing thinness of the oceanic crust. The exploration of the ocean floors, which is becoming one of the major achievements of our time, is passed over in silence. A certain amount of continental drift is admitted, but with the proviso that it must have ceased at an early stage of our planet's history, shortly after the birth of the Moon, which stripped away the granite layer from the region that is now the Pacific basin. Not a word about palæomagnetism. Not a word about the hundreds of miles of relative movement along the San Andreas and other great faults. Not a word about the discovery that these movements have been speeding up—on average—during the last 100 m.y. or more and that we are now living in a phase of exceptionally intense geological activities. Instead, Gamow thinks "we are now living during one of the relative lulls in activity". The cause of Earth movements and mountain building is still referred to "the steady cooling and shrinking of our globe". Not a word about phase transformations and the exciting possibility that the Earth is expanding.

So one could go on. Prof. Gamow has failed to realize that the earth sciences are progressing at an ever-accelerating rate. New knowledge is continually disrupting parts of the old framework, and the geologist has to assess the bearing on his subject of the interplay of almost daily discoveries in many branches of science besides his own. Prof. Gamow has simply not taken the trouble. It is not good enough to take a pot-boiler that was thoroughly bad from the start, revise a few pages, stick on the date 1959, ignore all the unprecedented advances made since the end of the War (except for the age of the Earth) and then to claim that "the revised edition embodies the currently accepted theories", which the author believes are "now here for good". I am sorry to be so severe, but a reviewer's first loyalty must be to his subject. I know that Prof. Gamow could do very much better if he would take the necessary trouble and then re-write the book and have it examined as he goes along by a competent geologist. Perhaps he will be inspired to make amends in this way by a pregnant remark made by Igor Stravinsky in 1958: "We all feel we are right; but we felt the same way twenty years ago and today we know we weren't always right then"

Arthur Holmes

ACHIEVEMENT IN THE GLASS INDUSTRY

Pilkington Brothers and the Glass Industry
By T. C. Barker. Pp. 296+20 plates. (London: George Allen and Unwin, Ltd., 1960.) 40*s.* net.

THE Pilkington glass enterprises began in 1826 when William Pilkington joined his brother-in-law Peter Greenall and two other men in the purchase of a small glass-works in St. Helens. This works manufactured window glass by the crown process, that is, the spinning of an opened-out bulb on the end of an iron so that a flat circular disk was formed. The disk was of uneven thickness, the centre part where it had been held on the iron being the bull's-eye sold cheaply at the time, but now sometimes made specially to provide a false appearance of antiquity. Soon William's elder brother joined him, and in 1829 the two bought out the other partners. William Pilkington's business ability was of great importance; he toured the country making contacts and obtaining orders, and the building boom of the 1830's helped the young firm to prosper. To this day the Company remains a private, family one.

The activities of the Firm have always been marked by very sound judgment in the choice of developments which were worth backing. During a depression in the industry around 1840 the improved cylinder process for sheet glass, which their rivals Chance's had been developing for some years, was introduced and the initial troubles had been overcome when the market boomed once more. Much later, in 1921 the development of the continuous process of manufacture of plate glass was undertaken in co-operation with the Ford Motor Co., and Pilkington's were ready to meet the new market which came with the expanding car industry. This fascinating story of the Ford–Pilkington co-operation is told in appendix 8. In 1919, C. W. Avery began to experiment in Detroit with a continuous process for plate glass. In 1919, he wrote: "We sent for a little crucible from Germany. It was about a month before we got the crucible back and it only held about a pint". By December 1921 1,500,000 dollars had been spent and the progress made impressed Pilkington's representatives so much that agreement was reached for co-operation in the further development of the process. Pilkington's, with their extensive knowledge of melting glass in tank furnaces, were then able to make a very important contribution. The continuous grinding and polishing plant was first put into use in 1923. In this process a continuous ribbon of glass nine feet wide issuing over a weir from a tank furnace passes through the annealing lehr and then under the grinding heads. In 1935, the twin grinder, a Pilkington development, which grinds both sides of the ribbon glass simultaneously, began operation at Doncaster.

Although there were no formal research laboratories as such until 1938, the firm was well able to make use of technical development. Cecil Pilkington was an Oxford graduate in natural sciences: in 1901, assisted by Leonard Lackland, a graduate of Liverpool, he introduced recording pyrometers and with their aid reduced the annealing schedule from 4 days to 4 hours. Electrical power was introduced in 1893; vacuum sucker pads were first used for handling plate glass in 1913. This present year, 1961, will see the opening of the 250,000 sq. ft. laboratories at Lathom Park about ten miles from St. Helens. Curiously, the firm appears to have lagged behind in the development of sheet glass, that is, flat glass which is made by a process which produces sufficiently good surfaces to require no subsequent grinding or polishing. The developments in the Fourcault continuous sheet process in the early 1900's had been watched carefully, but in 1928 sheet glass was still being made in St. Helens by the machine-drawn cylinder process and the production had to be subsidized by other activities. In 1930 interest was shown in the Pittsburgh Plate Glass Company's sheet process and by 1933 all sheet glass manufactured at St. Helens was made by this process ; since that time Pilkingtons have made important developments in this process. An entirely new development announced a year ago is the 'float process' which produces continuously, without grinding and polishing, flat glass with the quality of plate glass, on the development of which £4,000,000 was spent.

The present book is primarily an economic history of the Company ; the rather random notes of developments noted in reading the book and referred to in the previous paragraphs show that there is also a most interesting outline of the technical progress. As a chapter in economic history the book appears well done. There are more than 780 references to original sources, including Board minutes and documents in the Company's archives. It is an impressive story of a family which for four generations has produced efficient and shrewd business and technical men ; enlightened employers, with time not only to build and run one of the largest industrial concerns in Britain but also to devote much of their time to public service. In a statement issued by the founders Richard and William Pilkington to the work-people during some labour troubles in 1845 is the following, "It is our duty and our study, and we have always considered it our interest, to promote the happiness and welfare of all . . .". The story stops before the present generation took office, but they are carrying on the traditions in a manner worthy of their forbears.

R. W. DOUGLAS

INDIAN MONOGRAPHS ON ALGAE

Cyanophyta

By Dr. T. V. Desikachary. (I.C.A.R. Monographs on Algae.) Pp. x+686. (New Delhi: Indian Council of Agricultural Research, 1959.) 72*s*

Zygnemaceae

By Dr. M. S. Randhawa. (I.C.A.R. Monographs on Algae.) Pp. 478. (New Delhi: Indian Council of Agricultural Research, 1959.) 50*s*.

THESE two books are concerned mainly with freshwater algae which, in Britain, are of economic interest only to those concerned with the supply and purity of water. When present in large numbers they are generally considered as pests. In other countries, notably more or less tropical ones, they form the basis for a supply of much-needed protein in the form of fish which feed directly on them or through food-chains the few links of which ensure a relatively efficient secondary production. They can also be used in a simple, effective process of sewage purification ('oxidation ponds') and may increase the efficiency of solar salt works. Several of the Cyanophyta described fix nitrogen and play an important part in the cultivation of rice. Since rice is the staple food of a large part of the human race, and poverty in nitrogen a common feature of tropical soils, such algae are of great economic importance. Lastly, the possibility of mass culture of algae for direct use as food is being studied by biologists in many countries. Therefore the Indian Council of Agricultural Research is to be congratulated on starting an ambitious series of monographs which will eventually cover all algal groups. English is the international language of much of Asia and Africa, while the world-wide distribution of most freshwater algae makes these books of value to all phycologists.

These first two volumes set a good standard for the whole series. They are well set out and printed, richly illustrated, contain few misprints or misstatements and their cost is reasonable. They are more than Floras because there are sections on structure, reproduction, biology and general classification.

The taxonomy and nomenclature of algae are today in a confused state. Therefore each author has had to decide which 'system' to follow or to provide his own. Both are experienced workers, and, in the main, they use the classical system exemplified by *Die Susswasserflora Deutschlands, Osterreichs und der Schweiz* and Rabenhorst's *Kryptogamenflora*, though systems advocated by other authorities are described. It is disappointing that they do not criticize these diverse viewpoints. The non-specialist may also believe that the species and genera are clearly understood when, for the Cyanophyta at least, there are very few for which this is the case.

The first 75 pages of Dr. Desikachary's *Cyanophyta* cover structure, reproduction, classification and biology, and make clear once again how ignorant we are about these algae, notably concerning heterocysts, gas-vacuoles and mucilage. The next 545 pages describe all the taxa known from India, including ten new to science. The book concludes with a list of about 800 references and a rather scanty index.

Matters which may be criticized are omissions of some of the ecological and physiological features of these algae, too little consideration of the important work on micro-anatomy based on the use of the electron microscope, and the statement that the separation of *Petalonema* from *Scytonema* "appears to be convenient" ; modern work shows that this is incorrect. There also seems to be a lack of appreciation of the strong probability that some species of *Dactylococcopsis* are in fact green algae (*Ankistrodesmus*).

Dr. Randhawa's account of the Zygnemaceæ is more ambitious, for it covers the flora of the whole world. As this is a smaller and more coherent group than the Cyanophyta such a treatment is easier, and it adds considerably to the value of the book. In the first 100 pages a general consideration of these algae is given on similar lines to that appearing in the volume on Cyanophyta. A useful feature here is the section on their distribution in India in relation to the seasonal climatic changes, though it would be preferable to have altitudes, temperatures and rainfall expressed in metric units. There follow some 300 pages of keys and descriptions of species with an index (there is no general index) and a good bibliography.

Valuable features are the author's own numerous records of the occurrence of the species in India, so that one knows he has studied them himself. It is a pity he has not made more original drawings, because some of the illustrations chosen are not very satisfactory. Dr. Randhawa, like most modern workers on the group, has little use for infra-specific categories. This is in marked contrast to the other major group of the Zygnemales, the desmids, where specialists describe innumerable varieties and forms.

Although the important work of Dr. M. B. E. Godward on the cytology of *Spirogyra* is mentioned (p. 21) it is ignored in the section on the nucleus (pp. 53–59); consequently, the taxonomic potentialities of nuclear and chromosomal structures are also ignored, as are the new species she has described. In the section on Lloyd's discovery of contractile vacuoles in the gametes of *Spirogyra*, Matvienko's finding that these may only appear in the male gamete is omitted. The maps are puzzling. Naturally, like most maps covering the distribution of freshwater algae, these show mainly where phycologists have worked, but some features are misleading. Thus there are apparently only two species of *Zygnema* and four of *Spirogyra* recorded for England, whereas, in fact, about eight times as many are recorded. It is correctly stated on p. 261 that *Zygogonium ericetorum* has been recorded from every continent, but reference to map *H* gives the false impression that it is unknown in Europe outside England, in Asia outside India and China, in Africa outside the Union of South Africa and in America outside the United States.

Such criticism of these books as seems necessary does not alter the fact that they are considerable achievements and deserve the wide circulation they are likely to obtain. J. W. G. Lund

EUROPEAN SPIDERS

Die Tierwelt Deutschlands und der Angrenzenden Meeresteile nach Ihren Merkmalen und nach Ihrer Lebensweise

Begründet von Prof. Dr. Friedrich Dahl. Weitergeführt von Maria Dahl und Prof. Dr. Hans Bischoff. 47 Teil: Spinnentiere oder Arachnoidea (Araneae). XI: Micryphantidae—Zwergspinnen. Von Dr. Hermann Wiehle. Pp. xi+620. (Jena: Gustav Fischer Verlag, 1960.) 107.65 D.M.

This volume completes a series of monographs on the German spider fauna which began in 1926 with Dahl's *Salticidae*. The group of spiders described in this volume, known to the British araneologists as the sub-family Erigoninae, has been elevated to family rank by Dr. Wiehle under the name Micryphantidae. The text covers 615 pages, has 1,147 figures and describes 144 species in 74 genera. Together with the previous volume on the Linyphiidae, Dr. Wiehle has completed a monumental work which will stand as an authoritative text on European spiders for many years. Apart from the British Ray Society monograph by G. H. Locket and A. F. Millidge published during 1951–53, the present volume is the only modern work dealing with the difficult taxonomy of a very large family of tiny spiders, of which more than half are between 1 and 2 mm. in length. The German fauna is of particular interest to the British student because it includes both western and eastern European species, whereas the British fauna (144 species in 68 genera) is predominantly western and northern. Adequate taxonomic works on the Linyphiidae and Micryphantidae have become especially important in recent years because of the development of ecological studies on spiders, particularly those species in the litter and ground vegetation, most of which are members of these two families.

Wiehle's subdivision of the Micryphantidae into four sub-families follows closely that of Petrunkevitch in 1928. Sub-families Masoninae and Peleoopsinae have been generally recognized since they were proposed by Simon in 1884, and Wiehle has preferred Simon's name Walckenaerinae for the third sub-family instead of Petrunkevitch's Gonatiinae. The fourth sub-family, Erigoninae, remains, although there has been a redistribution of genera between it and the Walckenaerinae.

The genera and species keys owe a great deal to previous work by J. Denis in France and A. F. Millidge in England. The numerous figures are clearly drawn and an invaluable aid to identification. The list of references includes 187 titles, but omits that of Engelhardt (1958) which is referred to on page 1. Eric Duffey

NEUROSURGERY IN WAR-TIME

Neurosurgery

Vol. 2. Edited by R. Glen Spurling and Barnes Woodhall. (Medical Department, United States Army. Surgery in World War II.) Pp. xxvi+705+12 plates (283 figures). (Washington, D.C.: Office of the Surgeon General, Department of the Army, 1959. For sale by the Superintendent of Documents, U.S. Government Printing Office.) 7 dollars.

The previous excellent volume on neurosurgery in this series dealt with the organization of the specialty in the United States Army Medical Service during the Second World War and with head injuries; the present, and larger one, deals with spinal and peripheral nerve injuries.

The section on spinal injuries deals with every aspect of missile wounds of the spinal cord, with particular emphasis on details of nursing care, organization of evacuation and the urological problems presented by these patients. In the latter connexion, it is of interest that closed vesical drainage with manual control was preferred to automatic (tidal) drainage. An excellent account is presented of the work carried on in the paraplegic centres established in the United States to care for these terribly handicapped men, and the description of the financial and other assistance provided to resettle them in their homes and at work is impressive. The goal of those in charge of these centres is well summarized in the aphorism "Having added years to the lives of traumatic paraplegics, we must now, above all, add life to their years".

Peripheral nerve injuries are considered fully from anatomical, physiological and surgical points of view. Other chapters deal with injuries of these nerves combined with serious soft tissue, bone and vascular damage. Causalgia and its treatment are fully discussed. A most valuable chapter correlates the clinical features with the pathological changes, gross and microscopic, occurring at the site of injury in a series of more than 200 peripheral nerves.

In both this volume and that which preceded it one is constantly aware of the tremendous organizing ability which provided and so successfully staffed the large number of neurosurgical centres in the United States and abroad during the Second World War. This organization went further, however, in uniting the centres into one great team with the experience, therapeutic advances and research findings at one centre being made known to all through bulletins, meetings and the visiting consultants. The advantages of such an organization over that existing at the time in Britain need no emphasis—here, large numbers of neurosurgical (military and civilian) casualties were cared for in civilian hospitals, by neurological surgeons in some, orthopædic surgeons in others, each group working in relative isolation, some with an inadequate staff and without facilities for research or discussion of their problems with other groups interested in the field. While such a state of affairs was of course not invariable, it did exist. Not the least valuable contribution of these volumes from the United States could be the guidance they might provide concerning the organization of war-time neurosurgical services in Britain should the need for this again arise. J. E. A. O'Connell

BERYLLIUM

Beryllium

By G. E. Darwin and Dr. J. H. Buddery. (Metallurgy of the Rarer Metals, No. 7.) Pp. ix+392. (London: Butterworths Scientific Publications, 1960.) 70*s*.

POSSESSING, as it does, low density, high-elastic modulus, moderate strength and good oxidation resistance, beryllium would be an attractive structural material were it not for its low impact strength and high notch sensitivity. Because of these limitations, beryllium has been important mainly as a minor addition to alloys based on other metals, until the advent of nuclear energy made new demands on the metallurgist and necessitated a re-appraisal of the so-called rarer metals. Because beryllium has low atomic weight and very low absorption cross-section for thermal neutrons, both the metal and its oxide, beryllia, are suitable for use in reactors.

Intensive research and development work in recent years has made possible the use of beryllium for the canning of nuclear fuel elements. Much work had to be done before this stage was reached, however, and the book now under review gives a timely summary of available knowledge. All aspects of the metallurgy of beryllium are discussed including the occurrence of beryllium-bearing minerals; the extraction, production and fabrication of the metal; its physical, mechanical and chemical properties; its alloying behaviour and its nuclear properties. A chapter is devoted to beryllia, another to the "Health Hazards and Their Control" that have complicated recent work on both metal and oxide, and a useful appendix is devoted to the determination of oxygen in beryllium.

A monograph on a single metal is generally expected to refer to every relevant paper of note, and the authors have clearly borne this in mind. References are numerous throughout, frequently with just a short sentence about each paper, and there are occasions when more critical comment would have been welcome. As might be expected, this applies mainly to those parts of the book which are concerned with aspects of the subject outside the authors' personal experience. The sections more directly concerned with the production and fabrication of beryllium for nuclear energy, and with the properties of most direct relevance, are written in a much more authoritative manner, and the volume will be welcomed by all those working in this field.

If only the problem of brittleness could be overcome, beryllium would be of the greatest interest to aircraft designers. Those aeronautical engineers who are already discussing the potentialities of the metal should welcome this comprehensive treatment, and they should find the chapter on the ductility of beryllium of special interest. The book is nicely produced and well illustrated and is a useful addition to metallurgical literature. A. G. Quarrell

RARE-EARTH CHEMISTRY

The Chemistry of Yttrium and Scandium

By Dr. R. C. Vickery. (International Series of Monographs on Inorganic Chemistry, Vol. 2.) Pp. vii+123. (London and New York: Pergamon Press, 1960.) 40*s*. net.

IF the purpose of a monograph is to present the reader with a concise, critical and systematically presented résumé of knowledge in a particular field, and to spare him so far as possible the need to refer to the original literature, this book is unsuccessful. Scandium and yttrium are commonly discussed together with the lanthanons, even though in all their properties they display the behaviour expected of elements in Group IIIA of the Periodic Table. It is, in fact, impossible to consider the chemistry of yttrium, in particular, without continual comparisons with rare-earth chemistry, and, except at the level of detail and completeness found in Gmelin's *Handbuch*, it is debatable whether the elements justify a book to themselves.

Following a rather diffuse discussion of the history, natural occurrence and atomic properties of both elements, the author sets out to treat the isolation and the chemistry of each in turn. In matters that touch on Dr. Vickery's extensive practical experience of rare-earth chemistry—the (curiously old-fashioned) treatment of their minerals, the extraction and the analytical chemistry of scandium and yttrium—the reader is overwhelmed with facts and details of procedure. Other chapters that should clearly set out the chemical and physical properties, crystal structures and thermodynamic data for their compounds are sketchy, confusing in arrangement and, in parts, apparently ill digested. Plates, diagrams and textual statements are too often quite uninformative, and the whole lacks a certain coherence. Much of it might have been written thirty years ago, and even such separation procedures as ion exchange receive a disproportionately small allowance of space. On the credit side the author has assembled a considerable (but not a complete) bibliography, though the reader's confidence in the reliability of references may be shaken by other evidence of exceptionally careless proof-reading and preparation of the manuscript. Counting obvious typographic errors, mis-spelt names, omission of units and downright factual blunders, I noted corrections on at least 29 of the 116 pages of text. A purist would want to alter the lamentable English style on many more. The book has a limited usefulness. J. S. Anderson

Haldane of Cloan

His Life and Times, 1856–1928. By Dudley Somner. Pp. 448+12 plates. (London: George Allen and Unwin, Ltd., 1960.) 42*s*. net.

In writing this new life of Lord Haldane, Mr. Somner had access to much material not available to Sir Frederick Maurice; but though his book gives a vivid and well-balanced picture of Haldane it does no more than General Maurice's book to solve the enigma of Haldane, and throws scarcely any fresh light on his achievements, particularly in those fields of most interest to the scientist. Of Haldane as an administrator, an educationist or a philosopher we are shown, in fact, less rather than more: the *Machinery of Government* report is dismissed all too briefly, and the reader of Mr. Somner's book is given no indication of the extent to which Haldane's thought has influenced subsequent investigations into the organization of government. The new material has gone rather to expand the political picture of the times in which Haldane moved, and of his later years particularly we are told singularly little.

Mr. Somner frankly acknowledges his debt to General Maurice, but the whole structure of his book is on different lines, without, however, contributing anything fresh, except perhaps new light on particular episodes or the general political background. Much of Haldane's thought, not only in administration but also in education, and more especially university education, and on the organization of research, for example, is, however, remarkably relevant to the issues of to-day, and what one would have looked for in any new life of Haldane on this scale is some reassessment of the significance of his thought and work in such fields. No hint is given, however, of Haldane's part in stimulating the institution of the University Grants Committee, or of the research grants of the Department of Scientific and Industrial Research, although his work for adult education is recognized. To the scientist who has any knowledge of Haldane's work or thought in such fields it may well seem that an invaluable opportunity has been missed of re-examining critically Haldane's work and thought as a strategist, as an educationist, as an administrator or as a philosopher in a context which is as relevant to the issues of to-day as a generation ago. It is in such a context, moreover, that the stature and achievements of such a man as Haldane are most apparent R. Brightman

Trans-Antarctic Expedition, 1955–1958

Scientific Reports. No. 4: Tides at Shackleton, Weddell Sea. By J. G. D. Pratt. Pp. 21+2 plates. (London: Trans-Antarctic Expedition Committee, 22, Gayfere Street, S.W.1, 1960.) 12*s*. 6*d*.

A British contribution to tidal knowledge in the Antarctic is always an event, and *Tides at Shackleton* is no exception. Piquancy is added to the article not only by the comparatively unusual method of observation—by Worden gravimeter on a freely floating ice shelf—but also by the refreshing and often humorous method of presentation. The ingenuity required to improvize and maintain such a sensitive instrument under the physical conditions encountered, so as to obtain a sufficiently long series of observations for analysis, is greatly to be admired, as also is the painstakingly careful treatment of the data. After having analysed these data by standard methods of tidal analysis, and obtained results of which any tidologist would be proud, the author then proceeds to devise what he calls a 'least squares' method of analysis (all standard methods of tidal analysis are fundamentally based on least squares theory) and produce harmonic tidal constants seriously in discord with the 'standard' results. Pratt gives his reasons for this as being the existence of spurious oscillation with periods near 24 hr. and 40 hr. But while a spurious diurnal oscillation can seriously affect the diurnal constants in a tidal analysis, neither this nor the 40-hr. oscillation could affect the phase-lag of M_2 by 75°. Rather than force what are believed to be transient oscillations into the mould of harmonic constituents by a least-squares method, it would be better to examine the residuals obtained after subtracting a predicted tide (from the results of tidal analysis) from observations. There must be an error in the 'least squares' analysis for M_2 (and probably also for O_1), and, in view of the paucity of tidal data in the Weddell Sea and Mr. Pratt's own sterling efforts, this should be exposed.

J. R. Rossiter

Industrial Fatty Acids and Their Applications

Edited by E. Scott Pattison. Pp. vi+230. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1959.) 56*s*. net.

This book is edited by Mr. E. Scott Pattison, manager of the Fatty Acids Producers' Council, New York, and consists of a series of contributions by senior consultants of a number of major chemical firms in the United States on the industrial production and main usages of the varied chemical derivatives of fatty acids.

Some of these products have an annual commercial utilization of many millions of pounds; their value as detergents, plasticizers, food additives, cosmetics, etc., are clearly and concisely described. Special mention is made of the recent increased commercial interest in 'tall oil', a rosin/fatty-acid by-product of the paper-making industries. The purification, chemical nature and usages of derived fatty acids are given extensive treatment in the text, as also are the industrial processes involved.

The interactions of fatty acids with varied chemical reagents are given detailed treatment and chemical equations are employed extensively to illustrate the fundamental reactions occurring.

This book will prove of great interest to the chemists and chemical engineers who wish to keep abreast of modern development in the industrial field of fatty-acid technology. It should also prove of absorbing interest to the academic organic chemist.

D. T. Lewis

Space Research

Proceedings of the First International Space Science Symposium, Nice, January 11-16, 1960. Edited by Dr. Hilde Kallmann Bijl. Pp. xvi+1195. (Amsterdam: North-Holland Publishing Company; New York: Interscience Publishers, Inc., 1960.) 170*s*

The First International Space Science Symposium was held at Nice in January 1960, under the auspices of the Committee on Space Research. The volume under review thus has a historic character as well as being an exceptionally well-edited proceedings of the symposium printed and bound to the same high standard.

An account of the symposium was given in *Nature* on June 4, 1960, so no more than the section headings will be given here: (1) "The Earth's Atmosphere", (2) "The Ionosphere", (3) "Tracking and Telemetering", (4) "Solar Radiation", (5) "Cosmic Radiation", (6) "Interplanetary Dust", (7) "The Moon and the Planets". Radiation from celestial sources is included under "Solar Radiation". The 100 papers cover mostly the two years of space research from the launching of the first artificial satellite to the time of the conference. Since another symposium is planned by the committee for next spring, there can be little doubt that this volume is just the beginning of an important series, and it is to be hoped that the future conferences and their proceedings will enjoy the same success as the first.

R. L. F. Boyd

Health Physics in Nuclear Installations

Symposium organised at the Danish Atomic Energy Centre, Risø, 25–28 May, 1959. Pp. 410. (Paris: Organization for European Economic Co-operation, European Nuclear Energy Agency; London: H.M. Stationery Office, 1959.) 2,500 francs; 37*s.* 6*d.*; 6 dollars; 21.30 D.M.

This volume contains thirty-two papers delivered at a conference on health physics in nuclear installations, held at Risø, near Copenhagen, in May 1959, under the auspices of the Organization for European Economic Co-operation and the Danish Atomic Energy Commission. The purpose of the conference was partly to introduce the subject to those entering this field, and partly to permit the presentation and discussion of new results. Accordingly there are several introductory papers on such subjects as the biological effects of ionizing radiations, the basis of the maximum permissible levels of exposure, and measuring techniques.

These are followed by papers on the control of radiation-levels and of the contamination of personnel. Practical problems of health physics and criticality control in specific types of installation, including reactors, chemical plant, and high-energy accelerators, are discussed. There is a final section on emergency situations.

All the papers are by acknowledged experts from the United States, Great Britain and Europe, and are authoritative and well produced. This is a valuable record for those who attended the conference. However, for those new to the subject, it does not quite take the place of a well-organized text-book, and, since practically all the information it contains can be found in the technical literature, it is of limited use to experts. The volume will probably be a useful addition to many libraries.

W. J. Whitehouse

Absorption Spectra in the Ultraviolet and Visible Region

Edited by Dr. L. Lang, in collaboration with Dr. J. Szöke, Dr. G. Varsányi and M. Vizesy. Vol. 1: Pp. 414. Vol. 2: A Theoretical and Technical Introduction. Pp. 80. (Budapest: Publishing House of the Hungarian Academy of Sciences; London: Collet's Holdings, Ltd., 1959.) 137*s.* 6*d.*

These are the first two volumes of a series in which "subsequent volumes will contain spectra of foreign authors and the collection will thus emerge from its strictly national environment and assume an international character". Volume 1 includes the spectra of some two hundred assorted substances, mostly in solution, in the form of graphs of log ε against wave-length supplemented with tables of selected values of log I/I_0 at different wave-lengths for specified concentrations. The volume contains separate substance and formula indexes, but no attempt has been made at classification. It is impossible briefly to indicate the nature of the substances considered—which range from complex compounds of cobalt through substituted benzenes to phenanthrenes, flavones and xanthins. These are presented in loose-leaf: unhappily, the file containing the sheets of the review copy was not strong enough for its job.

Volume 2 consists of a "theoretical and technical" introduction. The text is some forty pages long. It will scarcely help those who know some spectroscopy already, and it is, naturally, too condensed to be of much value as an introduction. It is written in English which is nearly always intelligible but often odd. The discussion of the emission from a hydrogen lamp is not only odd but also incorrect.

The preparation of a reference set of ultra-violet spectra presents an enormous task, in which the first step is to adopt a practical classification. It is indeed useful to have the Hungarian results collected together, but this random sample seems unlikely to serve as a basis for the ultimate catalogue of these spectra.

R. F. Barrow

An Introduction to Industrial Mycology

By George Smith. Fifth edition. Pp. xvi+399. (London: Edward Arnold (Publishers), Ltd., 1960.) 40*s.* net.

The publication in 1938 of *An Introduction to Industrial Mycology* by Mr. George Smith was a marked contribution to the study of moulds. That the book has now reached its fifth edition is evidence of how well it has been received. The text of the fifth edition includes descriptions of a few genera and species of fungi which were not previously included, and the relevant literature of systematic mycology which has appeared since the publication of the fourth edition in 1954 has been considered.

The major portion of the book is devoted to descriptions and illustrations of most genera of moulds which regularly occur on industrial products. This section is of considerable value to students of general mycology especially because of the attention devoted to members of the Fungi Imperfecti and particularly to species of *Penicillium* and *Aspergillus*. The remainder of the book provides a most useful introduction to beginners in mycology, since it deals with laboratory equipment and techniques including microscopy and photomicrography. Mr. Smith is to be congratulated on presenting the subject-matter in a most readable form with complicated terminology reduced to a minimum. One of the delightful features of the text is the manner in which the author draws on his rich store of experience and gives practical advice on techniques and staining which is invaluable to newcomers in the field.

Almost all the illustrations are from original photomicrographs and, being of high quality, add greatly to the value of the book. As compared with the previous edition, one figure has now been omitted; but replacements of four plates and new illustrations of eight species are included. The publishers have maintained the high standard shown by the previous edition.

John Colhoun

NATURALLY OCCURRING ALPHA-ACTIVITY OF DRINKING WATERS

By R. C. TURNER, J. M. RADLEY and PROF. W. V. MAYNEORD
Physics Department, Institute of Cancer Research : Royal Cancer Hospital, London, S.W.3

THE present article represents a continuation of investigations into the nature and levels of naturally occurring α-activity in man and his environment. We have demonstrated the great variations of α-activity to be found as between different human foods[1]. Following the observations on foods it was natural to study the radioactivity of drinking waters supplied to the population of Great Britain.

Several investigators[2-8] have reported measurements of the radium-226 content of potable waters in other countries, but very few data have been published during recent years on the activity of waters in Britain. Following the observations of J. J. Thomson[9] early in this century on Cambridge water, there was considerable interest in this subject, but much of the earlier work leaves doubt as to whether the observed values applied to radium-226 or also included gaseous products such as radon-222, known to exist in certain waters at considerably higher levels of activity than its parent. In the present experiments we have measured the radium-226 content of 71 drinking waters available in Britain, and in addition we report the values for radon-222 and radium-224 (thorium *X*) when present.

In most instances the water samples have been made available to us by the co-operation of the local water undertakings at the request of the Ministry of Housing and Local Government

Experimental Procedure

The water samples were selected from several points of view, including the size of population supplied and the nature of the associated geological formations. The specimens were chosen with the view of assessing the total daily intake of natural radioactivity throughout Britain from its water supplies.

Preliminary experiments indicated that, if polythene containers were used for the collection and transport of the samples, it was unnecessary to add either carrier or acid agent to the water at the time of sampling. No residual α-activity could be detected on the walls of such containers even after they had housed specimens of relatively high activity for several days.

A litre of each specimen of water to be measured was evaporated to dryness in a 'Vitreosil' beaker and the α-activity of the residue measured, using the α-ray counting technique previously described[10]. In a few cases where the mineral content of the water was very low, 20 mgm. of barium chloride having known low α-activity was added to the water before evaporation in order to provide adequate residue.

Measurements were made on each specimen of residue immediately after evaporation and at intervals of a few days during the following 30 days, by which time the radon-222 and its α-emitting daughters polonium-218 (radium *A*) and polonium-214 (radium *C'*) would have reached equilibrium with the radium-226 present. After 30 days no further change of α-activity of the residue could be detected in any of the specimens. Assessment of the amount of radon-222 above the level required for equilibrium with the radium-226 present in each water was effected by observations on a liquid sample 2–3 mm. deep, sealed in contact with a zinc–cadmium sulphide phosphor of area 93 cm.[2]. Adsorption by zinc sulphide crystals of the daughter products of radon-222 present in solution had been previously reported by Rosholt[11], who used the method to obtain quantitative yields of lead-218 (radium *B*) and polonium-214 (radium *C'*).

It has been observed by us that, when water containing dissolved radon at a level of activity many times higher than its content of radium-226 is assembled in such an arrangement, the α-counting-rate increases for the first hour after sealing the sample and thereafter decreases with the half-life of radon-222 (3·8 days), until the activity has fallen to the level represented by the content of radium-226 and other long-lived activity. If at any intermediate stage the water is removed and the activity of the phosphor itself observed, the counting-rate decreases rapidly during the first 10 min. or so, corresponding to decay of polonium-218 (radium *A*), and thereafter falls with a half-life of approximately 40 min. compatible with the decay of lead-214 (radium *B*) and bismuth-214 (radium *C*). It is thought that the solid decay products of the dissolved radon are deposited on the surface of the zinc sulphide crystals and the α-particles emitted by polonium-218 (radium *A*) and polonium-214 (radium *C'*) are assumed counted with 2π efficiency. So long as the water itself is present, the observed activity is maintained with the half-life characteristic of radon, although the radon α-particles themselves may contribute little to the counting-rate since their range in the medium is only 1 or 2 per cent of the depth of the water layer.

Fig. 1 shows the characteristic pattern of decay of α-activity observed in a water sample containing radon greatly in excess of the amount required for equilibrium with its radium-226 content. Each estimate of radon content has been corrected for decay between the time of collection of the sample and the actual measurement.

In a number of the more active water residues, fast pairs of α-particles were observed, indicating the presence of at least the shorter-lived members of the thorium series[10]. This conclusion was borne out by the subsequent variation of the thorium series activity with time, since it decreased rapidly during the first 20 days or so following evaporation, after which the thorium activity remained constant at a relatively low level. The presence of radium-224 (thorium *X*) in American potable waters has already been reported by Stehney[2], who found the levels of radium-224 in freshly sampled waters comparable with those observed for radium-226. Due to the short half-life of radium-224 (3·64 days) and of its α-emitting daughter members of the thorium series, this α-activity decreases to very low values in the 2–3 weeks subsequent to sampling. There is little doubt that the decreasing thorium series activity observed

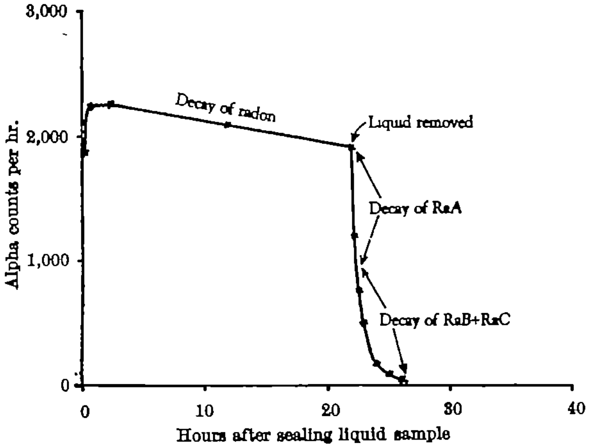

Fig. 1

in a number of our specimens is due to the presence of radium-224 and its daughters in the freshly sampled waters. In the absence of long-lived members of the thorium series, such as thorium-228 (radiothorium-228), at comparable levels of activity, the initial thorium series activity of these waters is regarded as due to radium-224 and its daughters. These values are given in Table 3 corrected for decay of activity since time of sampling.

In a number of the waters having very low activity it has not been possible to determine either the level of radium-224 or of the long-lived members of the thorium series.

In a few specimens having intermediate values of long-lived α-activity, the presence of thorium-228 and its daughters has been observed but at levels representing small fractions of the total long-lived α-activities given in Table 1.

Results

The values of long-lived α-activity together with the activities due to excess radon-222 and its daughters are given in Table 1 for various categories of water.

In a number of instances waters from more than one source have been measured for a given town, and these are listed separately. Ground-waters have been divided into two groups comprising those derived from boreholes in chalk and those from boreholes in other geological strata. Surface waters include sources such as springs, rivers, lakes and reservoirs.

Column 4 of Table 1 gives the content of solid matter observed (in parts per million), and column 5 the long-lived α-activity of this residue 30 days after evaporation of the liquid specimen.

Table 2 indicates the range of values and mean long-lived α-activity for the different categories of water and their residues.

In Table 3 the total α-activity of a number of the more active samples is broken down into its three main components.

Discussion

It will be seen from Tables 1 and 2 that the highest values of long-lived α-activity are found in the group of spa waters, which possess high contents of mineral matter having specific activities greater than those

Table 1

Origin	Long-lived α-activity (μμc./litre at 30 days)	Excess radon and daughters (μμc./litre at time of sampling)	Solid matter observed (parts per million)	α-Activity of residue (μμc./gm. at 30 days)
Spas				
*Vichy Celestin	48·5	—	3,150	15·4
Juventus	45·5	—	1,250	36·4
*Vichy Catalan	43·3	—	1,700	25·5
Bath	37·0	1,000–2,000	2,540	14·7
Llandrindod	26·2	200–300	3,320	7 9
Leamington	16·7	200–300	10,200	1·7
Buxton	4 5	1,500–2,000	210	21·5
*Vittel	2·2	—	675	3 8
*Perrier	1·8	—	370	4·8
Cornish waters				
St. Ives 1	9·40	9,000–10,000	163	57·6
Helston 3	4·10	600	170	24·1
Helston 2	2·20	100–200	125	17 6
Helston 1	1·41	1,000–2,000	83	17 0
Penzance 2	1·24	700–800	54	23·0
Penzance 1	1·02	100–200	70	14·6
St. Ives 2	1·00	9,000–10,000	42	23·8
Penzance 3	0·90	500–600	133	6·8
Camborne 1	0 59	4,000–5,000	83	7·1
Camborne 2	0 46	4,000–5,000	5	92·0
Ground-waters (not chalk strata)				
Bristol 1	2·90	300–400	1,230	2·4
Wolverhampton	2 28	—	273	8·4
Bristol 2	1·75	600–700	300	5·8
Leamington	1·60	100–200	420	3·8
Spalding	1·34	100–200	360	3·7
Hartlepools	1·00	100–200	380	2·6
Ashford (Kent) 2	0 58	~ 10	210	2 8
Cirencester	0·38	—	290	1 3
South Staffs 1	0·37	—	183	2·8
Ground-waters (chalk strata)				
London 1	1·22	—	380	3·2
Uxbridge	0·65	—	216	3·0
Canterbury	0·30	—	234	1·3
Sutton (Surrey)	0·27	10–20	100	2·7
Ashford (Kent) 1	0·26	—	200	1 3
Portsmouth	0·23	—	209	1·1
Colne Valley	0·22	100–200	360	0·6
Amersham	0·22	—	217	1·0
Barnet	0·19	100–200	200	1·0
Farnham Common	0·11	—	125	0·9
Brighton	0·11	—	188	0·6
Surface waters				
Bristol 3	0·81	100–200	370	2·2
Glamorgan 1	0 62	300–400	240	2·6
Bristol 4	0·52	50–100	158	3 3
London 3	0·47	—	380	1·2
London 2	0·41	—	284	1·5
Bath	0·33	10–20	244	1·4
Belfast	0 33	—	30	13·1
Paignton	0 30	—	25	12·0
Blaenau Festiniog	0·20	—	—	—
Skipton	0 20	< 1	80	2·5
Aberdeen	0·14	—	42	3 3
Manchester 1	0·12	—	54	2·2
Charminster	0 11	—	96	1·2
Dundee	0·11	< 1	33	3 3
Torquay	0·11	< 1	55	2·0
Edinburgh	0 10	< 1	33	3·0
Aberystwyth	0·10	—	—	—
Glasgow	0·09	—	—	—
Glamorgan 2	0 09	—	38	2·7
Sheffield	0 09	—	54	1 7
South Staffs 2	0·08	—	208	0·4
Hamilton	0·08	< 1	38	2·1
Birmingham	0·07	—	33	2·1
Manchester 2	0·05	—	29	1·7
Argyll	0·05	< 1	15	3·3
Elan Valley	0·04	—	5	8·0
Caernarvon	0·04	—	—	—
Manchester 3	0 04	—	21	1·9
Dolgelley	0·03	—	30	1 0
Bangor	0·03	—	—	—
Betws-y-Coed	0·03	—	—	—
Hazleton	0·02	—	—	—

* Bottled specimens as purchased

Table 2. LONG-LIVED α-ACTIVITIES IN DRINKING WATERS AND IN THEIR RESIDUES

Type of water	No. of samples	Activity of water in $\mu\mu$c./litre			Activity of residue in $\mu\mu$c./gm.		
		max.	min.	mean	max.	min.	mean
Spa	9	48·5	1·8	25·1	36·4	1·7	14·6
Cornish	10	9·4	0·46	2·2	92·0	6·8	28·4
Borehole (not chalk)	9	2·9	0·37	1·4	8·4	1·8	3·7
Borehole (in chalk)	11	1·2	0·11	0·34	3·2	0·6	1·5
Surface	32	0·8	0·02	0·18	18·1	0·4	3·2

Table 3 α-ACTIVITIES IN $\mu\mu$C./LITRE

Specimen	Short-lived α-activity due to radium-224 (Th *X*) and daughters at time of sampling	Long-lived α-activity after 30 days	α-Activity due to radon-222 and daughters at time of sampling
Llandrindod Spa	30·0	26·2	200–300
St. Ives 1	21·0	9·4	9,000–10,000
Bath Spa	7·7	37·0	1,000–2,000
Bristol 2	2·9	1·8	600–700
Helston 2	2·8	2·2	100–200
Helston 3	2·4	4·1	~ 600
St. Ives 2	0·7	1·0	9,000–10,000
Penzance 3	0·5	0·9	500–600
Camborne 1	0·3	0·6	4,000–5,000
Penzance 1	0·3	1·0	100–200
Camborne 2	0·2	0·5	4,000–5,000

found for surface waters or waters derived from boreholes.

The Cornish specimens, although having a mean level of activity lower than the spa waters by a factor of 12, are still approximately ten times higher in activity than the mean values of chalk borehole or surface waters. The mineral contents of the Cornish waters are relatively low; but the mineral matter has specific activities ranging up to 92 μμc./gm., doubtless associated with the known deposits of uranium and radium in parts of that county.

Next in descending order of long-lived α-activity come the drinking waters derived from boreholes in geological strata other than chalk. The mean activity of this group is only slightly lower than that found for the Cornish waters, and this would appear to be due to their higher mean contents of mineral matter, 400 parts per million compared with 90 for the Cornish samples.

Waters derived from boreholes in chalk have still lower activities with a mean level down by a factor of 4 below those derived from boreholes in other strata. It is interesting to note that the mean value found for the α-activities of their mineral residues (1·5 μμc./gm.) is very close to that reported previously by us[12] for the α-activity of chalk specimens.

The least-active group of waters observed by us are surface waters from rivers, lakes and reservoirs, even though a number are derived from areas of Pre-Cambrian or granitic rock formations. Their mean mineral content is 100 parts per million and their mean long-lived α-activity is lower by a factor of 12 than the Cornish waters.

There was, of course, the possibility that a substantial fraction of the observed long-lived α-activity was due to uranium-238. However, measurements of uranium contents of a number of waters in Great Britain have been reported by Smith and Chandler[13], who found values of less than 1 μgm. (0·34 μμc.) per litre in 124 out of a total of 162 specimens examined. Waters with values higher than this figure were, with one exception, not drinking waters. Our own observations on the growth of α-activity of the residues during the 30 days following evaporation leave little doubt that also in our series of waters the content of radium-226 is several times higher than the activity due to uranium-238.

Moreover, investigations of the uranium contents of North American river waters by Rona and Urry[14] show a ratio of radium-226 to uranium-238 of approximately 4 : 1. In another study of the activities of ground waters in the United States, Scott and Barker[15] also observed levels of radium-226 in excess of those due to uranium-238.

These results are borne out by the chemical estimations made by us of the uranium-238 contents of a number of our more active residues. These confirm the presence of uranium at levels of activity which represent only a small fraction of the total long-lived α-activity in each case. It is to be noted that even if the radium/uranium ratio is as low as 4 : 1, then the α-activity due to radium-226 and its daughters will still comprise 90 per cent of the total long-lived activity contributed by members of the uranium series.

In respect of the thorium series, Stehney[9] found no evidence of long-lived members such as thorium-228 in his studies of American potable waters, while Rona and Urry[14] assessed the ratio of thorium-230 (ionium-230) to uranium-238 in North American river waters as being a very small fraction of unity. The very low levels of long-lived α-activity due to the thorium series found by us in the present investigation agree with the findings of these observers.

In the absence, therefore, of either uranium-238 or thorium-228 at levels of activity comparable with those due to radium-226, the long-lived α-activities listed in Table 1 may be regarded as due to radium-226 and its α-emitting daughters, radon-222, polonium-218 and polonium-214, existing in radioactive equilibrium. The radium-226 content of any particular specimen is therefore taken as one quarter of the long-lived activity given in column 2 of Table 1. It will be seen, therefore, that the contents of radium-226 observed in this series of waters cover a range of 2,400–1, namely, from 0·005 μμc./litre to 12·1 μμc./litre.

If we exclude the spa waters and assume a daily intake of 2·5 litres per head of the population of Britain[16], then the daily intakes of radium-226 from water alone cover a range of almost 500–1, namely, from 0·013 μμc. to 5·9 μμc. In terms of long-lived α-activity the intakes from water range from 0·05 to approximately 24 μμc. per head per day.

So far we have considered only the long-lived α-activity due to the presence of radium-226 and its daughters. It will, however, be seen from Table 1 that a number of the waters possess relatively very high contents of radon-222 and its daughters at the time of sampling. This is especially the case in the Cornish specimens where the α-activities due to radon-222 and its daughters, polonium-218 and polonium-214, range up to 10,000 μμc./litre. On the basis of a daily intake of 2·5 litres the ingested α-activity can therefore be as high as 25,000 μμc. a day, that is, approximately 8,000 μμc. of radon and of each of its two short-lived α-emitting daughters. In any attempt to assess the possible significance of these materials we are faced with an almost complete lack of data concerning their metabolism in the human body. Meyer[17] in a classical series of experiments in the late 1920's came to the conclusion that radon ingested in drinking water had a 'mean life' of about 1 hr. in the body, being excreted principally by the lungs. On this assumption it is easy to calculate that during its stay in the body approximately 0·75 per cent, that is, 60 μμc. of radon and of each of its two α-emitting daughters, disintegrate completely. Since 1 μμc. of radon-222 comprises 1·78 × 10^4 atoms of radon, this implies the production of about 3 million α-particles and of a million or so atoms of lead-210 (radium *D*) in the body as the result of each day's intake of such water. In a future paper it is hoped to discuss certain aspects of the radiation dosimetry resulting from these findings.

It will be seen from Table 3 that a number of waters at the time of sampling also contain radium-224 (thorium *X*) and its three α-emitting daughters at levels of activity comparable with those of the long-lived activity due to radium-226 and its daughters. Since radium-224 is an isotope of radium-226 their fundamental chemistry would be identical, but the short mean life of radium-224 and its decay products may result in a very different distribution of absorbed energy.

Daily Intakes of Alpha-Activity

We now consider the mean daily intakes of α-activity per head of population resulting from drinking water. From information kindly supplied by the Ministry of Housing and Local Government we have estimated the number of consumers of each of the principal water supplies. Assuming a daily intake of 2·5 litres per head the mean daily intakes of α-activity from this source for various sections of the population are estimated in Table 4.

Table 4. MEAN DAILY INTAKE OF α-ACTIVITY FROM DRINKING WATER μμc. PER HEAD OF POPULATION

Group of population	Long-lived activity Ra-226 and daughters +	Activity radon and daughters	+ Activity Ra-224 and daughters
Cornwall	4·5	8,300	~ 5·5
London area	1·0	20	very small
Whole of Britain	0·5	17	,, ,,
Scotland	0·25	2	,, ,,
Spa patients (1 litre per day)	21·1	~ 1,000	—

It will be seen that the mean daily intakes of long-lived α-activity for the four groups cover a range of 18 to 1, whereas from Table 1 it is clear that the range of individual intakes may exceed 400 to 1. For the mean daily intakes of radon plus daughters we have a range of 4,000 to 1 between the groups, with the range for individuals exceeding 10,000 to 1. The low mean value of long-lived α-activity in the Scottish waters illustrates, rather surprisingly, the low values which may be encountered in areas of granitic formations. For comparison, Table 4 also shows the approximate daily intake of α-activity resulting from the consumption of 1 litre per day of British spa water having an activity equal to the mean value observed by us for this type of water.

Maximum Permissible Levels of Natural Activity

The report of the International Commission on Radiological Protection[18] deliberately excludes 'natural background' from its scope. It is, however, recommended by the International Commission that, in considering the exposure of large populations in relation to somatic effects, the maximum permissible level be taken as one thirtieth of the continuous occupational value (168 hr./week) computed according to its basic rules. The value of maximum permissible concentration for soluble radium-226 in water suggested by the International Commission for continuous occupational exposure is 100 μμc./litre, so that the value for large populations would be 3·3 μμc./litre. This value is lower than our observed values for British spa waters and two Cornish waters. The activities of several other waters are more than 10 per cent of this estimate. A similar situation has been noted for some large populations in the United States[19].

However, direct observations by us[10] on human skeletons from Cornwall show that the radioactivity observed in them is many times less than would be expected on the basis of the assumptions made by the International Commission. The observed mean level of activity of the skeletons is a factor of at least a thousand below the lowest body burden known to have resulted in a tumour.

The apparent anomaly arises largely because the biological parameters which have been used in the calculations were based on observations on human subjects having large radium burdens acquired many years previously and who were therefore excreting the radioactive material at a very low rate.

In a previous article[20] we reported investigations of the levels of natural α-activity present in the daily diets of the population of Great Britain and came to the conclusion that the daily intakes of such activity could vary from a minimum of 4 or 5 μμc. to 400 or 500 μμc. or even more, depending on the particular choice of diet. It is evident that in places where the drinking water has a level of α-activity at the higher end of our observed range and the individual intake from food happens to be at the lower end of the range of dietary activity, then the daily intake of natural α-activity will be dominated by the contribution from water. In other locations where the drinking water is derived from surface sources having much lower activity, the contribution from food will be predominant, regardless of the choice of diet.

Whether the human body finds it easier to retain radium-226 and its daughters present in soluble form in drinking water than to retain the same elements, possibly in different chemical form, ingested in food, is not yet known. The relative contributions made by water and food to the real retention and utilization by the body of α-active substances and the factors which control retention in the two cases are the subject of present investigations.

Comparison with Fission Product Activity in Waters

Finally, perhaps we may consider these daily intakes of natural α-activity in water in relation to the published figures of the artificial β- and γ-activity due to the presence of strontium-90 and cæsium-137 in drinking water in Great Britain[21].

The pattern of distribution of these fission products is quite different from that of the natural α-activity, since they appear at their highest levels in surface waters. Ground waters have extremely low contents of fission products, but, as we have seen, high values of natural α-activity.

From the latest figures available (for the first half of 1959) we calculate the mean contents of strontium-90 and cæsium-137 in surface waters as 0·75 $\mu\mu$c./litre and 0·20 $\mu\mu$c./litre, respectively. The mean daily intakes of populations ingesting such drinking waters would therefore be 1·9 $\mu\mu$c. and 0·5 $\mu\mu$c. of the two nuclides, respectively.

For large populations the International Commission on Radiological Protection suggests 33 $\mu\mu$c./litre and 6,000 $\mu\mu$c./litre in water, respectively, for these materials as the maximum permissible concentrations. It will be seen that the observed values are very small in comparison to these permissible levels.

Note added in proof. Since these investigations were completed, we have had the opportunity of examining specimens of drinking waters supplied to Horrabridge, Devon. The radioactivity of these particular waters has been the subject of recent communications[22,23] concerning the incidence of cancer in the area. We find the long-lived natural activity of the Sampford Spiney supply to be 0·6 $\mu\mu$c./litre, and its content of radon + daughters 2,500–5,000 $\mu\mu$c./litre. It will be seen that these values are well within the range reported in Table 1 above for waters in south-west England.

Many of the water samples were obtained through the goodwill and co-operation of Mr. A. W. Kenny, of the Ministry of Housing and Local Government, who kindly approached on our behalf the chief engineers of numerous water undertakings, to all of whom we would like to express our sincere thanks.

Our thanks are also due to Mr. J. Crookall, of this Department, for chemical estimations carried out on a number of the samples.

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FIRE RESEARCH*

By S. H. CLARKE, C.B.E.

THE continuous systematic study of fire protection started in Europe and the United States in the closing years of the last century. Added impetus came with the Second World War, and modern fire research owes much to the vision of the late Sir Reginald Stradling, chief scientific adviser to the Ministry of Home Security, to Lord Falmouth, head of the Fire Research Division, and to the knowledge and experience of Prof. G. I. Finch and Dr. D. T. A. Townend. Prof. Finch suggested critical experiments which were of great value in elucidating some of the more important phenomena. He showed that, although many of the problems of fire protection were extremely intricate, they were in fact the result of a pattern built on relatively simple principles.

For a fire to occur there must be fuel, oxygen, and a source of ignition; there are exceptions, but they are of almost negligible practical importance.

It is helpful to approach the study of fire by examining a shallow dish of petrol—a volatile, flammable liquid. If a small flame is gradually brought near the surface the vapour ignites when the flame is perhaps an inch from the liquid; the eye can just follow the rapid spread of flame. Incidentally, a cigarette can be extinguished by gently dipping the lighted end into a tray of petrol.

If the experiment is repeated with a tray of a less volatile liquid, say paraffin (kerosene), it will be found that quite a large flame must be played directly on the surface for a short time before ignition takes place. Once ignition occurs, however, and the liquid is warm, if the flames are snuffed out by placing a board over the tray, immediate re-ignition occurs when a fresh flame approaches the surface, exactly as with petrol.

It is thus necessary to distinguish two temperatures; first, the 'flash-point', which is the temperature at which vapour distils rapidly enough to support a flame; and, secondly, the 'ignition temperature'—and this is usually a higher one—at which the flammable vapours can be ignited.

Although the chemical processes that are involved when wood is burned are much more complicated, in some ways wood and other similar materials may be thought of as behaving like liquids with a high flash-point. When wood is heated, it decomposes, and gases and vapours are formed which, in contact with air, behave just like those of petrol or paraffin, and burn with flame.

G. I. Finch summarized very neatly the behaviour of wood as a fuel. He found it convenient to classify combustible materials in three grades which he called "tinder", "kindling" and "bulk fuel". Tinder can be ignited by an ordinary match and is then able to continue burning; it has a specific surface of not less than 20 cm.^{2}/gm.; paper or wood shavings are an example. Kindling cannot be ignited by a match but can by tinder, and, if it is suitably arranged, can continue to burn; it has a specific surface of not less than 2 cm.^{2}/gm. Bulk fuel can only be ignited by a

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* Substance of a discourse at the Royal Institution on October 21.

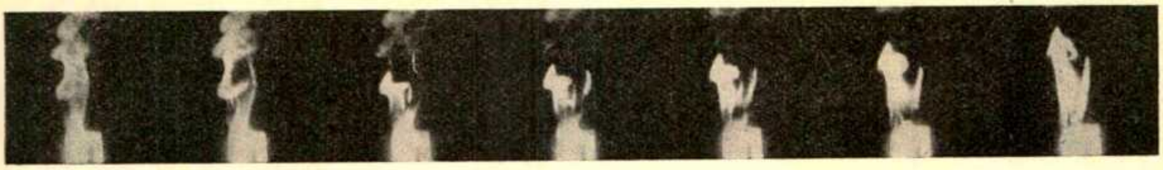

Fig. 1. Successive stages in the ignition of wood by radiation. Pictures taken at about 0·01 sec. exposure. Note ignition occurs above the specimen outside the radiation field. By courtesy of the Director of the Fire Research Station. Crown copyright reserved

fire which already involves kindling, but under suitable conditions it can sustain self-combustion; its specific surface is less than 2 cm.^{2}/gm.

P. H. Thomas and D. L. Simms observed an interesting feature in the burning of wood. When small specimens are subjected to radiant heat of high intensity, vapours and gases are emitted which ignite spontaneously. The interesting thing is that ignition occurs not at the point of applied heat but some distance above, and the flame jumps back rapidly to the wood (Fig. 1).

It should be noted that it is inaccurate to speak of a liquid burning; combustion occurs in the vapour phase, and in a region where there are convenient proportions of vapour and oxygen. When wood burns, about half the mass is consumed as flame and about half glows as charcoal.

The processes described form the background to the early stages of most fires. Once started, a fire spreads until all the exposed surface of combustible material is involved in flame. At that moment the maximum temperature is reached. The rate at which it is reached depends largely on the amount and arrangement of the combustible materials. When it is reached, subsequent developments depend largely on the access of air to the fire.

There are two basic principles of practical fire protection, namely, the control of possible sources of ignition, and the control of the amount of combustible material that can be involved at one time. The latter frequently involves the division of large buildings into compartments each of which has a pre-determined degree of fire resistance.

Observations of the temperatures reached at many large fires, and in experiments, have led to the almost universal acceptance of a standard time-temperature curve which is considered to represent the conditions in a severe fire. 'Fire resistance' is defined as the length of time for which a unit of structure continues to perform its design function when it is heated in a furnace which is controlled according to the standard curve. By-laws in most countries now require buildings in towns to be constructed so that their walls, floors, structural columns, and certain doors and windows reach an appropriate degree of fire-resistance. Steel columns have a fire resistance of about ten minutes, and the problem is, therefore, to provide them with a heat-resisting coating which will stay in position when involved in fire.

It sometimes comes as a surprise to find that steel behaves so badly in fire; but it may be a greater one to learn that a number of fires have been caused by the combustion of fine steel-wool, which is readily ignited by a match, or even by allowing a handful to fall across the terminals of a small battery; and there have been many fires in boilers in which overheated steel tubes have burned in high-pressure steam.

The variety of possibilities in constructing fire-resistant buildings is so great, and the present state of our knowledge so small, that full-scale tests are often necessary before approval can be given for the use of a new form of building construction. Furnaces were built by the Fire Offices Committee nearly thirty years ago to enable such tests to be carried out, and they were immediately made available to the Building Research Station of the Department of Scientific and Industrial Research for research purposes. When the Joint Fire Research Organization was set up in 1947, they formed part of the contribution of the Fire Offices Committee to this Organization.

Fig. 2 shows a specimen after test, and gives an idea of the size of the operation. Such tests are costly and time-consuming, and much research has been designed to find means of eliminating them, or at least of conducting them on a smaller scale. The work has involved the search for mathematical expressions for the variations in temperature in every part of a wall, floor, column, etc., when the temperature at the surface is raised at the rate demanded by the standard time-temperature curve. The construction of these may be elaborate, and even when the thermal constants of the constituents are known the calculation of rise of temperature in the steel is a difficult

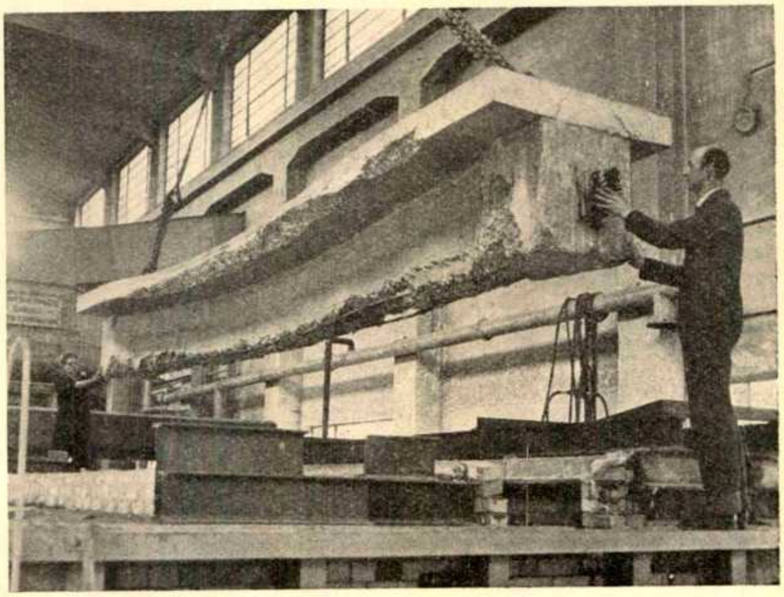

Fig. 2. Full-size specimen of pre-stressed concrete beam after fire resistance test. By courtesy of the Director of the Fire Research Station. Crown copyright reserved

and tedious operation. Lawson and McGuire devised and constructed an electrical analogue which has proved extremely valuable both as a research tool and in reducing the number of full-scale tests. It exploits the close analogy which exists between the flow of heat in solids and the flow of a charge along a transmission line composed of series resistance and shunt capacitance elements.

The analogue deals with one scientific problem, namely, the behaviour of units of building construction under closely defined conditions. There remains a wide range of problems resulting from the movement of hot gases and the transfer of heat, such as the provision of escape facilities in high buildings, the control of smoke in large basements or in ships, the 'mushrooming' of hot gases in buildings of large area, the detection of fire, and many aspects of extinction. Before these can be studied on a small scale, there is need of a reliable technique for the use of models. Considerable progress in this direction has been made by P. H. Thomas and C. T. Webster. Working with similar cubical models, of 1–10 ft. side, they have studied the burning of wooden cribs of various sizes, and have established relationships that have already enabled them to deal with some practical situations.

It has long been recognized, for example, that, smoke is perhaps the fireman's greatest enemy. It offers particular difficulties in buildings with a large expanse of floor area with all kinds of obstructions which together make it impossible for the fireman to see or to find his way to the seat of the fire. The Covent Garden fire and several other recent large fires in Great Britain have focused attention sharply on this problem. They have led to a revived interest in a method of fire protection that has been employed in theatres for many years, namely, the provision of emergency venting in the roofs to release the smoke and hot gases at an early stage in the fire. P. H. Thomas has recently developed for the first time what appears to be a satisfactory solution with an adequate theoretical basis. Using models, he has explored the combined use of 'curtains' to restrict the lateral spread of smoke in conjunction with roof-vents of various sizes, and has established a relation between vent area and the proportion of heat released in experimental conditions.

Detection

Experience of large fires soon convinces that, when they get beyond a certain size, total destruction is inescapable. A surprisingly large proportion of the total damage is caused by a relatively small number of large fires; each year nearly half the damage in the United Kingdom is caused by some 200–300 out of some 50,000 fires in buildings attended by the fire brigades. Of the many factors that contribute to the development of large fires the most important is failure to detect and attack them while they are still small. P. Nash and R. Pickard have explored the rise in temperature and the movement of hot gases over fires of various sizes in enclosures of various heights and shapes, a task which called for entirely new instruments for measuring air-flow. Using the results of this work, together with information about the distance of travel of fire brigades and experience of the desirable balance between high sensitivity and the likelihood of false alarms, they have succeeded in developing a standard procedure which enables the merits of new designs of detector to be assessed.

Before leaving the subject of the occurrence of fires, reference should be made to the fact that effective statistics are collected with the collaboration of the fire brigades of the local authorities, by arrangement with the Home Office. These have yielded a number of interesting results, including indications of significant differences in behaviour of pre-war and post-war houses and 'pre-fabs'—the post-war housing having the best record. They have revealed a steady increase in fires associated with electrical equipment, and with the use of certain types of oil-burning apparatus.

Extinction

When attempts to control sources of ignition and accumulations of fuel have failed, there remains the task of fire extinction. The most common method is to cool the fuel to a temperature at which the flammable vapours cease to be emitted.

The commonest extinguishing agent is water, and the principles that govern its use have not changed fundamentally over hundreds of years. Whenever it can be used, it is incomparably the best medium. There is plenty of it and it is cheap; it is easily transported (by pipes) and easily applied; it has a large thermal capacity and it boils at a convenient temperature, absorbing a useful amount of heat in doing so. The only circumstances in which plain water is not the best medium are electrical fires, fires involving flammable liquids the flash-point of which is below atmospheric temperatures, and circumstances in which water damage must be avoided at all cost.

The main lines of research in recent years have aimed at improvements in methods of applying water. Since the War improvements in equipment, training, and technique have resulted in a marked and continued steady improvement in the effectiveness of fire brigades in the United Kingdom.

In the early post-war years the opinion was held in some quarters that high-pressure sprays, which were believed to have very fine drops, represented the ultimate in efficiency. A long and careful study by D. Hird, in which the fire brigades in London and Birmingham took part, demonstrated that there is no material advantage in increasing operational pressure much above about 100 lb. per sq. in., and any theoretical merit fine drops may have in extinction is offset by their limited trajectory.

D. J. Rasbash has shown that, where water is used, fires in liquids which cannot be cooled below their flash-point can be extinguished in the vapour phase by the use of a very fine spray. Less-volatile liquids such as fuel oil can be extinguished by cooling, and larger drops which will pass through the flames and reach the surface of the liquid are more effective.

Another way of using water is as foam, which is made by dissolving a foaming agent (often a protein derivative) in water and aerating the solution mechanically, or by producing bubbles of carbon dioxide chemically. The function of the foam is to cover the surface of the liquid and to shield it against radiant heat from the fire, thus cutting off the supply of flammable vapour.

During the War it was found possible to introduce foam at the base of a tank of fuel oil, and to let it rise by its low relative density and spread over the surface of the oil. This was not possible with petrol, because in ascending through the bulk of the liquid the foam became impregnated with petrol, which continued to burn and destroyed the foam. Recent work has led

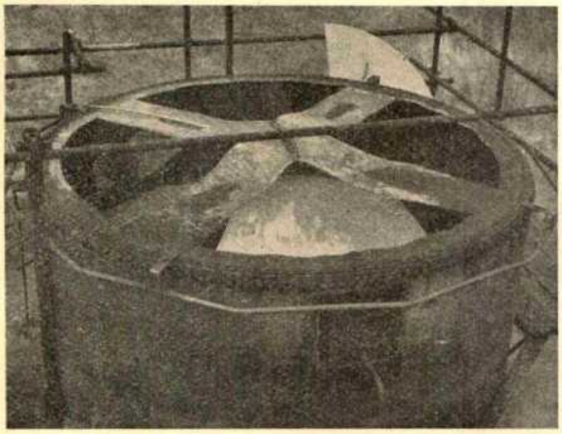

Fig. 3. Last stages of extinction in petrol fire by foam injected at the base of a tank 30 ft. high. By courtesy of the Director of the Fire Research Station. Crown copyright reserved

to the surprising conclusion that very sloppy foams, with an expansion of about three times, are effective even with petrol (Fig. 3).

Two other types of extinguishing agents are used for special fires. The first are known as vaporizing liquids, the commonest being carbon tetrachloride. They operate in the vapour phase by absorbing heat, and possibly also by inhibiting combustion. Research has been devoted to seeking to understand the reactions involved, and to seeking the most effective of a wide range of reagents. Taking into account cost, convenience, toxicity of the agent or its decomposition products, chlorobromomethane has come to be regarded as the best of the available materials.

D. J. Rasbash recently made the bold suggestion that the principle of controlling the atmosphere might be used on the grand scale even for such fires as that at Covent Garden. A large jet-engine was specially modified by the National Gas Turbine Establishment, and the exhaust gases from this engine are being used experimentally in a large building to see how far practical use can be made of the principle. The inert, exhaust gases do not cool the fuel, but it is hoped that they will prevent the spread of combustion long enough for a fireman to be able to approach with his hose.

The other type of medium is known as 'dry-powder', the commonest form being sodium bicarbonate. Research has not yet fully elucidated its mode of action, but experience has thrown considerable doubt on its superiority over other cheaper and better-known methods.

Fire as a Social Problem

Fire is a social problem. An example will illustrate the complications that are encountered in applying the results of research. A few years ago D. I. Lawson and his colleagues studied the occurrence of casualties through the ignition of clothing. Fire brigades and a number of hospitals co-operated by sending reports on a large number of incidents, and when possible they sent samples of the fabrics which were first ignited. The researches involved studies of a large range of fabrics in the laboratory, and a simple equipment was developed as a standard measure of their flammability. Statistics showed that nearly a quarter of the fatal casualties were children, and of those a large proportion were wearing nightdresses. Very few occurred between the ages of about 10 and 60, and the vast majority were elderly people and often old ladies. It became clear that the practical solution was not to aim immediately at the prohibition of all but flame-resistant fabrics ; there was need for special care of the young and old, who might wear such materials, and increased attention to the control of sources of ignition such as electric fires, gas fires, and the domestic fire. It was considered important that the public should be informed about the special hazards of certain fabrics and that means should be provided for them to be able to recognize the ones which presented the least hazard.

Fire touches practically every aspect of life, and deliberations of this kind call for wide collaboration. Some fourteen Government Departments in Britain have special concern in fire protection : the Home Office is responsible for the safety of the public where they assemble in buildings and other places, and for ensuring that the efficiency of the fire brigades is maintained : the Ministry of Transport for safety in ships : the Ministry of Aviation for aircraft, and so on. The Fire Research Board gives opportunity for members of industry and of the universities to consider their problems and, with assessors from the Government departments, to ensure that the research programme is balanced and co-ordinated.

The Joint Fire Research Organization itself is believed to be the only example of complete and equal partnership of Government (through the Department of Scientific and Industrial Research) and industry (through the Fire Offices Committee) in arranging at national level for research to deal with a social problem.

OBITUARIES

Prof. Erwin Schrödinger, For.Mem.R.S.

Erwin Schrödinger, who died on January 4, was a man with outstanding ability to apply abstract mathematical reasoning to the advancement of physical theory. His great discovery, namely, Schrödinger's wave equation, as the basis of the description of atomic processes, was one of the most surprising of all the sudden advances that have occurred with the development of scientific knowledge.

Schrödinger was born in Vienna in 1887. He was a student at the University of Vienna during 1906–10, and then acquired a mastery of eigenvalue problems in the physics of continuous media, so laying the foundation for his great work. He became assistant at the University of Vienna, and later served as an artillery officer during the First World War.

In 1920, having married Annemarie Bertel, he went to Jena to take up an academic position. This was followed by positions at Stuttgart (extraordinary professor), Breslau (ordinary professor), and then the University of Zurich, where he settled for six years. Throughout this period he was very actively engaged in research in theoretical physics and published many papers, mostly on the specific heats of solids and other questions of thermodynamics, and on atomic spectra.

He has told me how he came to make his great discovery. With his work on spectra he was using, of course, Bohr's orbit theory, but he always felt that the quantum conditions in this theory were unsatisfactory and that atomic spectra should really be determined by some kind of eigenvalue problem. In 1924, de Broglie published his work on waves associated with the motion of free particles. This influenced Schrödinger profoundly, and he set to work to try to generalize de Broglie waves to bound particles. He finally obtained a neat solution of the problem, leading to the appearance of the energy-levels as eigenvalues of a certain operator. He immediately applied his method to the electron in the hydrogen atom, duly taking into account relativistic mechanics for the motion of the electron, as de Broglie had done. The results were not in agreement with observation. We know now that Schrödinger's method was quite correct, and the discrepancy was due solely to his not having taken the spin of the electron into account. However, electron spin was unknown at that time, and Schrödinger was very disappointed and concluded that his method was no good and abandoned it. Only after some months did he return to it, and then noticed that if he treated the electron non-relativistically his method gave results in agreement with observation in non-relativistic approximation. He wrote up this work and published it in 1926, and in this delayed manner Schrödinger's wave equation was presented to the world.

A short time previously Heisenberg had discovered his matrix mechanics, another formalism for dealing with atomic processes. The theories of Heisenberg and Schrödinger were soon found to be equivalent, there being a mathematical transformation that converts one into the other. Two independent and apparently entirely different lines of thought thus led to the same foundation for atomic physics.

Schrödinger published a series of papers in quick succession, developing his ideas. For this work he shared the Nobel Prize in Physics for 1933.

In 1927 he unfortunately moved to Berlin, where he became Planck's successor. In consequence his life became disturbed by political developments, and with Hitler's coming to power in 1933, he decided he could not continue in Germany. He came to England, and for a while held a fellowship at Oxford. In 1936 he was offered a situation at Graz. He was well aware of the uncertain position of Austria at that time, and it was a difficult decision for him to make; but the attraction of returning to his native country outweighed his caution and he accepted.

With the German annexation of Austria in 1938 Schrödinger was immediately in difficulty, because his leaving Germany in 1933 was taken to be an unfriendly act. He was forced to express his approval of the Nazi regime, and he did this in as ambiguous a way as he could (see *Nature*, **141**, 929; 1938).

He soon found life with the Nazis intolerable, and, profiting by the fact that they had neglected to take away his passport in the first place, he was able to slip across the frontier to Italy. He proceeded to Princeton University and after a short stay there moved to Dublin, to the newly created Institute for Advanced Studies, where he became director and remained until his retirement in 1955.

All this time Schrödinger continued his research and published many papers on a variety of topics. Some of them were concerned with unifying gravitation and electromagnetism, the problem which absorbed Einstein for many years and which is still unsolved. Schrödinger remained greatly interested in the foundations of atomic physics. He did not like the generally accepted dual description in terms of waves and particles, with a statistical interpretation for the waves, and tried to set up a theory in terms of waves only. This led him into controversy with other leading physicists.

Schrödinger was unconventional in his way of life. When he went to the Solvay conferences in Brussels, he would walk from the station to the hotel where the delegates stayed, carrying all his luggage in a rucksack on his back and looking so like a tramp that it needed a great deal of argument at the reception desk before he could claim the room that had been reserved for him.

After his retirement he returned to an honoured position in Vienna. He died after a long illness. He is survived by his wife, who was his faithful companion throughout their marriage.

P. A. M. Dirac

Dr. Sidney Marsh

Dr. S. Marsh, who was head of the Physics Department of Battersea Polytechnic from 1919 until 1950, died on December 14, at the age of seventy-six.

After completing a double first in physics and mathematics at University College, Cardiff, he went to Munich and Göttingen, where he obtained his Ph.D. on work on discharge tubes under Prof. E. Riecke. He joined the staff of Battersea Polytechnic in 1909 and, except for the two years 1917–19, when he was head of the Mathematics and Physics Department at Rutherford Technical College, Newcastle on Tyne, he remained there until his death. He was appointed head of the Department in 1919, and, after his retirement in 1950, he continued as a part-time lecturer giving more than part-time service. He gave his last lecture on December 12, 1960, two days before his death. The way he continued to adapt himself to the rapidly changing conditions of work in the post-war period was very remarkable. Old regulations and new regulations, general and special degree, polytechnic and college of advanced technology, he took it all in his stride.

His research interests lay in electrolysis and gas discharges, but the passion of his life was teaching, and, while this goes unrecorded in the proceedings of learned journals, it lives on in the hearts and minds of all those that passed through his Department during his long stay there. With immense energy he also threw himself into the examination work of the University of London, and for many years right up to his death he was the mainstay of the external examinations in physics. Marsh must have been a name to countless students, the world over, who never knew of the immense pains he took to be fair to them and the compassion with which he carried out his duties as an examiner.

As a young man taking over what had been his Department for so long, I might have expected to find him a little difficult as a colleague. Far from this being so, he was a most kindly and considerate person who was never obtrusive with his advice, though always willing to give it. He had that true humility that is a token of true greatness, and those who have known him and have worked with him mourn his loss greatly.

L. R. B. Elton

Mr. H. T. Pledge, C.B.E.

By the death of Mr. H. T. Pledge on December 28, the library and scientific worlds have suffered a great loss.

Humphrey Thomas Pledge, son of Thomas Pledge, was born on November 6, 1903. He went to Tonbridge School and thence as an Exhibitioner to Trinity College, Cambridge, where he took a first in the Natural Sciences Tripos. After a short period of teaching at Wrekin College he entered the Science Museum Library as an assistant keeper in 1928.

During his early years in the Library, Pledge was concerned with its services to industry, both in lending and in providing information. It was during this time that he collected material for his major work, published by H.M. Stationery Office in 1939, under the title *Science since 1500*. This is a comprehensive and masterly historical survey of the development of the physical and biological sciences, which has been widely accepted as a standard book of reference. It has been reprinted three times in Great Britain and has also been published in the United States.

In 1945, Pledge became keeper of the Science Museum Library, and the full burden of the restoration of its services after their dislocation during the War fell on his shoulders. Later developments which added to his administrative responsibilities were the photocopying service and the impact on the Science Museum Library of the formation of the National Scientific Lending Library to take over gradually the former's lending service to industry. During his tenure of the keepership he continued to build up the Library into one of the finest scientific libraries in Britain, in spite of stringent limitations on the financial resources available to him.

Pledge's interests were wide, ranging from biography through the physical and biological sciences to ethnology and archæology, and his knowledge was deep. His book brought fellow workers on the history and philosophy of science from all over the world to South Kensington to visit him. He took an active part in the founding of the British Society for the History of Science.

In 1934, Pledge married Ruth, daughter of the Rev. H. M. Brown, and they had one daughter. He was made a C.B.E. in 1960.

S. E. JANSON

NEWS and VIEWS

U.S. Atomic Energy Commission: Dr. Glenn T. Seaborg

Dr. Glenn Theodore Seaborg, who has been appointed chairman of the U.S. Atomic Energy Commission, was born in Ishpeming, Michigan, on April 19, 1912. After graduating at the University of California in chemistry in 1934, Dr. Seaborg held various research and teaching appointments in that University until 1942, when he was appointed section chief in the Manhattan Engineer District's Metallurgical Laboratory at the University of Chicago, where he conducted research in nuclear chemistry and physics; here he was responsible for developing the process used for the separation of plutonium from fuel elements irradiated in the production reactors at the Hanford installation at Richland, Washington. In 1946 he returned to the University of California at Berkeley as a professor in the Department of Chemistry. He was appointed chancellor of the University in 1958.

While serving as instructor at the University of California in 1940, Dr. Seaborg was co-discoverer of element 94 (plutonium), the first of a number of trans-uranic elements which his research activities revealed over the next fifteen years. He was the discoverer in 1941 of the isotope used as a source of nuclear energy, plutonium 239; in 1942 of the uranium isotope, uranium 233; in 1944 of elements 95 (americium) and 96 (curium); co-discoverer in 1949 of element 97 (berkelium); co-discoverer in 1950 of element 98 (californium); in 1952 of element 99 (einsteinium); in 1953 of element 100 (fermium); and in 1955 of element 101 (mendelevium). He was a member of the Atomic Energy Commission's General Advisory Committee from 1946 until 1950; member of the Joint Commission of Radioactivity, International Council of Scientific Unions, since 1946; and of the Committee of Standards and Units of Radioactivity, National Research Council, since 1947.

Anatomy and Biology at Western Reserve University

Prof. Marcus Singer has been appointed Henry Willson Payne professor of anatomy in Western Reserve University, Cleveland, Ohio. He is at present professor of zoology and of child development and family relationships in Cornell University. Prof. Howard A. Schneiderman, associate professor of biology in Cornell University, has been appointed professor of biology in Western Reserve University. Both appointments will be effective July 1, 1961.

A native of Pittsburgh, Prof. Singer first graduated from the University of Pittsburgh, and later was awarded M.A. and Ph.D. degrees by Harvard University. He was on the Harvard staff during 1938–51, first in the Biology and Zoology Departments and later in the Anatomy Department of the Medical School. In 1950 he served as visiting professor at the Long Island College of Medicine in Brooklyn, New York. He went to Cornell as associate professor of zoology and of child development and family relationships in 1951 and became a full professor in 1953.

Prof. Schneiderman is a native of New York City. He attended Swarthmore College and Columbia University and graduated from the former in 1948 with honours in mathematics and natural sciences. He was granted the degree of M.A. and a doctorate by Harvard University. During 1952–53 he was a University research fellow in biology at Harvard. In 1953 he was appointed assistant professor of zoology at Cornell and was appointed associate professor in 1956.

Botany at Calcutta: Prof. S. M. Sircar

Dr. S. M. Sircar has recently been appointed Sir Rashbehari Ghosh professor of botany in the University of Calcutta. This chair, created in 1914 in the name of its donor, is the oldest in Indian universities for fostering research work in botany with special reference to agriculture. Dr. Sircar was educated at the Presidency College and the University College of Science, Calcutta. During 1934–36 he worked under Prof. F. G. Gregory, at the Imperial College of Science and Technology, London. After returning to India in 1937 he joined the University College of Science, Calcutta, and took active interest in organizing research in plant physiology, particularly with reference to the rice plant. During 1946–47 Dr. Sircar served as acting head of the Department of Biology in the University of Dacca and from 1949 until 1951 he was on deputation with the Government of India as professor of agricultural botany in the Central College of Agriculture, New Delhi. He has created a strong school of research in Calcutta with special reference to vernalization, photoperiodism, mineral nutrition, germination and auxin relations of the rice plant. In 1954 he was elected a Fellow of the National Institute of Sciences of India. In 1958 he was awarded a travel grant by the Rockefeller Foundation to visit various universities and research institutes in Europe, the United States and Japan. The appointment of Dr. Sircar to the chair is a fitting recognition of his long association with the University of Calcutta and his continued interest in the rice plant.

School of Management Studies, University of Bristol

The University of Bristol is to establish a School of Management Studies for the purpose of providing residential courses in management studies and for conducting research into problems of business management and administration. This has been made possible by an offer by the Foundation for Management Education to underwrite the sum of £14,500 a year for a period of seven years in support of the School, and by the agreement of the University Grants Committee to supplement the Foundation's support with a contribution of its own. The initial programme for the School will be the offer of a residential course for executives in the 28–34 age group, who have attained departmental responsibilities and who are nominated by their employers as being suitable for the kind of training which the School will provide. The course will be of eight weeks duration, divided into two periods of four weeks each, these periods being separated by an interval of about ten weeks, during which the students concerned will be expected to undertake specified studies within their own employing organization. It is intended that the session 1961–62 will be occupied as a preparatory year for the School and its first admissions should be in the autumn term of 1962. Further information can be obtained from the Registrar, University of Bristol, Bristol 8.

Scientific Literature on Fire

The tenth volume of *References to Scientific Literature on Fire* has recently been published by the Fire Offices' Committee, Joint Fire Research Organization (Library Bibliography No. 5/Part X. By Miss E. M. Shakeshaft and Mrs. B. F. W. Rogowski. Pp. 107. Boreham Wood, Herts: Fire Research Station, 1960). This contains 1,117 title references to papers, most of which appeared in 1956, arranged under eight headings. The largest section (311 references) is entitled "Initiation and Development of Combustion", and refers mainly to original and fundamental studies. Other sections are devoted to branches of fire technology—occurrences of fire (103 references), fire hazards (106), fire precautions (149), fire resistance (178) and fire fighting (229). Short sections headed "Atomic Energy" (18 references ranging from radioisotopes to nuclear weapons) and "General" bring the volume to a conclusion, except for extensive name and subject indexes which occupy as much as two-fifths of the whole publication and have evidently been compiled with much care and thought. There is no doubt that this series fulfils a useful purpose, especially as regards the fire technology references, most of which would not be found in other bibliographies.

Royal Society Committee on Scientific Research in Schools

The Committee on Scientific Research in Schools which was established by the Council of the Royal Society in 1957 has issued a report covering the period November 1, 1959–October 31, 1960. It is stated that interest shown by schools in undertaking research has continued to increase, and the Committee is now administering research projects in 56 schools. Some of these schools have more than one research project under way and a total of 66 separate research projects are now being actively pursued compared with 57 last year. During the year, £2,000 has been provided by the Council of the Royal Society, and Shell Research Ltd. have given an additional £75 for special assistance at one school. All research projects with which the Committee is dealing are carried out with the specialist advice and assistance of Fellows of the Society and by others who act as advisers. The Committee invites further requests from school-masters and school-mistresses for assistance in undertaking research. A list of the schools at which research projects are being carried out and a list of the projects themselves—which cover a very wide range of topics in the physical and biological sciences—are given as an appendix to the report.

North Atlantic Biogeography

The American Geographical Society, working together with a panel set up by the Committee on Oceanography of the U.S. National Academy of Sciences—National Research Council, proposes to publish a scientific journal of an unusual kind, tentatively called *North Atlantic Biogeography*. The journal will consist of maps, accompanied by such explanatory text as may be necessary, and will appear irregularly as contributions are received and accepted. It will be a medium for publication of studies of all kinds—biological, geological, physical, chemical—that will illuminate our geographical understanding of the marine environment. Its scope will be limited to the Atlantic marine areas from the equator to the pole and will include the Arctic basin. The plan is sponsored in the United States by the National Academy of Sciences—National Research Council, and in Canada by the Royal Society of Canada. Funds are being sought in both countries. The panel is international. Further information can be obtained from the Director, American Geographical Society, Broadway at 156th Street, New York 32.

Botanical Garden, Allahabad

A new botanical garden has been established at Allahabad in the premises of the Central Botanical Laboratory, Botanical Survey of India. The garden is divided into various sections such as an economic plants garden, experimental garden, ornamental section, landscape section, lily pools, fern houses, orchidarium and nursery. The temperature and humidity are being manipulated in the fern houses and orchidarium by artificial fountains and green cover. A variety of species is being introduced in the different sections. The institution is establishing exchange relations for seeds and plants, and it may be possible in the near future to publish an inventory of the seed and plant material available. Gifts of seeds and plants will be welcomed by the Director, Central Botanical Laboratory, Allahabad, India.

Efficiency of Indexing Systems

THE report on the first stage of an investigation into the comparative efficiency of indexing systems issued in September 1960 (see *Nature*, 188, 276; 1960) has now been followed by an interim report on the test programme briefly outlined in that earlier report (Aslib Cranfield Research Project: *Interim Report on the Test Programme of an Investigation into the Comparative Efficiency of Indexing Systems*. (An investigation supported by a grant to Aslib by the National Science Foundation.) By Cyril W. Cleverdon. Pp. iii + 84. (Cranfield: College of Aeronautics, 1960.) 8*s*. 6*d*.). For this programme more than 1,400 questions were received, from which 400 were selected for the first round of testing, about half relating to aerodynamics. Although the test programme is still incomplete and the clerical analysis of such testing as has been done is unfinished, the general level of the four indexing systems is of particular interest and possible surprise. There also appeared to be only a small difference between the success of the postings for the short time of 2 min. as against the relatively long time of 16 min. Comment is also made on the ability of a relatively inexperienced person to index with an efficiency equal to that of the other more experienced members of the staff. Comments on the general level of efficiency are in this report based solely on finding the same document, and indicate that technical staff were able to use the indexes as efficiently as the project staff, the only significant difference being with the alphabetical subject catalogue. Technical staff with no previous experience got on surprisingly well with the Universal Decimal Classification, but in regard to the facet system opinion is provisionally expressed that the chain index and single order approach were a severe handicap and that use of the schedules in a more conventional manner would have given more satisfactory results. Both the project staff and technical searchers found the uniterm system relatively simple to use.

Calcutta School of Tropical Medicine

THE joint annual report of the Calcutta School of Tropical Medicine and the Carmichael Hospital for Tropical Diseases for 1958–59 contains a full record of the work of the scientific staff of the many departments. A number of matters of outstanding importance are referred to. Among these was the breeding of a strain of black mice by the crossing of white and ordinary mice; these were used for transmission of human leprosy bacilli, with promising results. A new experimental device has been evolved for research on amœbiasis. In an attempt to produce better vaccine for cholera, it is recorded that formalin, in a suitable concentration, appeared to preserve antigenicity better than other chemical or physical agents. In the field of virus research, an interesting observation has been made. While the vaccinia virus was invariably found to be lethal to infant mice inoculated intracerebrally, the variola virus was found to survive in the brain of mice for a prolonged period without producing any pathogenic effect. Intensive work continued on the hæmoglobinopathy syndrome, yielding results of considerable interest.

Medical Aspects of the Kariba Dam

A SPECIAL supplement of the *Central African Journal of Medicine* describes medical aspects of the Kariba Hydro-Electric Scheme (6, No. 10; October 1960). The paper has been prepared by Dr. M. H. Webster, formerly chief medical officer to the Federal Power Board, Kariba, who outlines the measures taken to deal with the health problems of the Kariba scheme stemming from the following factors: the topographical and meteorological features of the site; the nature of the undertaking; the importation of African workers in large numbers and at high speed from many parts of Central Africa; the importation of large numbers of European operatives who could be expected to have little or no immunity to the specific disease risks likely to be encountered and, indeed, as it turned out, little or no experience of tropical conditions. One of the first undertakings of the Board was a campaign, carried out from the air and on the ground, against the tsetse fly and mosquitoes which infested the area. As soon as possible an adequate hospital was built, but its modern equipment and comprehensive facilities would have been of little use without the skill and devotion of its staff. In a foreword, Sir Duncan Anderson, chairman of the Federal Power Board, states his belief that the study will prove a valuable source of information and guidance to all engineers and administrators who are concerned with the planning of great projects in the remote corners of the Earth.

The Quelea

IN many regions of Africa the quelea bird has always been a threat to agriculture, causing damage to crops, especially sorghum, millet, rice and other small-grained cereals. The economic losses have increased considerably during the past fifteen years or so, and to-day, in many African countries, more especially those of West Africa, all attempts to raise output are doomed to failure unless methods for the effective control of these birds are jointly elaborated and simultaneously applied. This was the problem before the symposium arranged by the Commission for Technical Co-operation in Africa South of the Sahara, held at Bamako during May 17–21 (*Science Afrique*, 21; July 1960). The agenda covered three main points: a review of progress achieved in control techniques—chemical, physical and biological means of destruction of roosts and breeding colonies; co-ordination at regional and interterritorial level—co-ordination concerning local migrations, ringing campaigns, direct exchanges of information on biology and control between neighbouring territories; control of birds other than quelea presenting a threat to crops. The participants were also called on to consider the possibility of developing joint action in Africa to combat this natural scourge. In their recommendations, they pointed out that progress in quelea control must depend on adequate financial means to apply really effective measures. The meeting also stressed the importance of direct exchanges of information between technicians working in this field, and more especially between technicians in contiguous regions. A ringing programme was recommended to include at least 100,000 birds over a period of three years, and a budget drawn up for such a programme. Another recommendation dealt with the need for investigations on the physiology and parasitic diseases of quelea as being indispensable in determining fresh control methods.

New Stomatopod Crustaceans in West Africa

In November 1956, Dr. D. T. Gauld, of the University College of Ghana, sent to the British Museum (Natural History) three small Stomatopods, collected at Labadi, West Africa. One of these proved to be an immature specimen of a *Lysiosquilla*; the other two specimens were labelled *Squilla pallida*, but this identification proved to be incorrect. Later, two further specimens identical with those submitted by Dr. Gauld were found among a collection of *Squilla africana* Calman from Accra. It was apparent that these four specimens belonged to an undescribed species of *Squilla* as they did not agree with any known West African species, or with the descriptions of any of the known Eastern Atlantic ones. The name *Squilla labadiensis* has been given to this new species, which is described by R. W. Ingle in the September issue of *Annals and Magazine of Natural History* (2, No. 21; 1960). During the course of describing *Squilla labadiensis*, Ingle re-examined several West African specimens of *Squilla* in the British Museum (Natural History) collections. In particular, among a collection presented to the Museum by Mr. A. R. Longhurst, it was discovered that several specimens labelled *Squilla mantis* had been wrongly determined, and these specimens were identified as *Squilla intermedia* Bigelow, hitherto known only from the Gulf of Mexico and Little Bahama Bank, and the range of which is now extended to the West African coast. The description of *Squilla intermedia* is included in the paper.

Canavanine in Leguminosae

The amino-acid canavanine is known to be present in the free state only in members of the Leguminosae. It occurs in the seeds and its synthesis has been reported in rooted leaves of *Canavalia* sp. Its presence can be detected by simple chromatographic techniques. B. A. Birdsong, R. Alston and B. L. Turner have now reported on a systematic study of the distribution of this substance in 161 species, representing 78 genera, of Leguminosae, and have discussed the impact of their findings on the phyletic grouping in this family. These results are supplemented by data, variously collected, by other authors, to yield a total of 219 investigated species representing 109 genera. Of these, 68 species belonging to 32 genera have canavanine in their seeds. Canavanine has not been found in the sub-families Mimosoideae or Caesalpinioideae. In the sub-family Papilionoideae it has not been found in the tribes Podalyrieae and Sophoreae. At least one species from each of the other tribes produces canavanine. In three genera, some species produce canavanine whereas others do not. These data are presumed to be of taxonomic significance and have been interpreted as support for chromosomal information, indicating that the tribes Mimosoideae and Caesalpinioideae diverged relatively early from the 'stem' line which is now represented by the Papilionoideae (*Canad. J. Bot.*, 38, 499; 1960).

New Nature Reserve

The Nature Conservancy has declared a new national Nature reserve of 1,363 acres at Invernaver, near Bettyhill, on the north coast of Sutherland. The reserve includes woodland, heath and sand-dunes. Its subsoil is limestone, and this brings in a number of rare and local plants, including the Scottish primrose *Primula scotica*, the mountain avens *Dryas octopetala*, and the purple mountain milk-vetch *Oxytropis halleri*. The attractive little spring squill *Scilla verna* also grows in the reserve, and there is a moss that grows nowhere else in Britain. The breeding birds of the reserve include the greenshank, red-throated diver and ring ouzel.

Symposium on Zooplankton Production

Following the success of the symposium on primary plankton production held in Bergen in 1957, it is proposed to hold a symposium in Copenhagen in 1961 entitled "Zooplankton Production". It will be held towards the end of the annual session of International Council for the Exploration of the Sea during October 8–10. The symposium is planned to include: two sessions on methods of estimating zooplankton, methods used at sea and in the laboratory, methods of assessment of standing crop and production; one session on the food of zooplankton, the trophic relationships of the organisms, and regeneration of nutrients; one session on behaviour, including vertical migration, distribution, speed of movement and zooplankton as a possible source of sonic scattering layers; one session left open for extra papers and discussion of recommendations. To keep within the schedule, and yet allow time for the very valuable discussions, the papers at the first four sessions will be by invited speakers only. Papers for consideration for the fifth session will be welcomed, at this stage by title only with a paragraph of explanation if this is not clear in the title. The organizing committee includes: Dr. J. H. Fraser (convener), Marine Laboratory, P.O. Box 101, Victoria Road, Aberdeen; Prof. E. Steemann Nielsen (Copenhagen); Prof. J. Krey (Kiel); Dr. Gunnar Thorson (Helsingør). Those who intend to be present should notify the convener as soon as possible.

"Educating the Whole Man"

In an article, "The Problem of Freedom and Efficiency in Universities", in *Science and Freedom* for October and in a broadcast talk, "Dry Rot and Redbrick", Dr. A. P. Rowe pleaded earnestly for a thorough reconsideration of the relations between teaching and research in British universities. He suggested a searching inquiry into the quality of both, as well as into the balance between them, and also that changes in the structure of the administration of the universities might facilitate the promotion of more effective and stimulating teaching, and the elimination of much of the trivial investigations which now pass for research. A sentence in the leading article in *Nature* of January 7 (p. 2) unwittingly misrepresents Dr. Rowe's mind. So far from rejecting any idea of a broad education, he is concerned that the universities of Great Britain are failing to provide that vital something that was once given by the homes from which their students came; and although he advocates that more research should be carried out in research institutes rather than in university departments, such institutes should be, he thinks, an integral part of the university.

D.S.I.R. Studentships, Fellowships and Research Grants: 1961

Two booklets, issued by the Department of Scientific and Industrial Research, have become available and give detailed information on grants to be offered by the Council for Scientific and Industrial Research during 1961 for studentships, fellowships and special research. The first, entitled *D.S.I.R. Studentships and Fellowships, 1961,* gives the information required by young scientists and technologists who wish to obtain grants in order that they may continue their training beyond their first degree (Pp. 28. London: H.M. Stationery Office, 1961. 1*s.* 9*d.* net). Information is also given of awards made available by the Council and tenable abroad, and of Nato science studentships and fellowships. In most cases it is required that applications for these grants be made by March 1. The second booklet, entitled *D.S.I.R. Research Grants, 1961,* gives information on grants for special research work (Pp. iv + 8. London: H.M. Stationery Office, 1961. 1*s.* net). This covers such grants as are applicable to investigators who are already of acknowledged standing in their subjects. Applications for these grants should be submitted to the Council by May 1 or November 1. Grants issued by the Department of Scientific and Industrial Research are essentially for British subjects normally resident in the United Kingdom, but in certain cases the Council will consider persons of other nationalities who are working in the United Kingdom.

Lady Tata Scholarships and Fellowships

The Trustees of the Lady Tata Memorial Trust invite applications for grants, scholarships and fellowships for research on diseases of the blood, with special reference to leukæmia, in the academic year beginning October 1. In view of the affinity between leukæmia and other forms of neoplastic disease, candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukæmia will be eligible for consideration. The Trustees specially wish to encourage studies of the leukæmogenic viruses in mammals, the epidemiology and natural history of leukæmia, and the genetical and cytological aspects. Further information can be obtained from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London, S.W.3. Applications must be submitted before March 31.

"Radiative Recombination in Gallium Phosphide Point-Contact Diodes"

Drs. H. C. Gorton, J. M. Swartz and C. S. Peet write: "The acknowledgment to the United States Air Force, which sponsored the work reported in the communication under this title published in *Nature*, 188, 303 (Oct. 22, 1960), through subcontract with the Lincoln Laboratory, Massachusetts Institute of Technology, was inadvertently omitted. Appreciation is hereby expressed to the Lincoln Laboratory for permission to publish this work".

"Labilization of Deoxyribonucleic Acid in Thymus Nucleoprotein after Whole-body Irradiation"

Referring to the communication under this title in *Nature*, 187, 1123 (1960), Dr. Ulrich Hagen writes: "In the text, Fig. 1 and in Tables 1 and 2, the extraction of DNA is described as being done with 'trichloracetic acid'. It must be emphasized that the sodium salt of trichloracetic acid was always used, at the concentrations mentioned (0·5, 1·0 ... per cent). The *p*H-value of the solution was 6·7".

Announcements

Prof. Theodore von Kármán, at the California Institute of Technology, has been awarded the 1961 James Watt International Medal of the Institution of Mechanical Engineers, in recognition of his contributions to the advancement of mechanical engineering science in the field of aeronautics. The award of the Medal is made every two years.

Dr. E. K. Janaki Ammal has been awarded the Indian Botanical Society's Birbal Sahni Medal. The Medal is awarded every year to a distinguished Indian botanist for his contributions and services to botany in India.

Volume 4, Part 4, *of Scientific Institutions and Scientists in Latin America*, issued by the United Nations Educational, Scientific and Cultural Organization, Scientific Co-operation Office, Montevideo, lists the scientists of Argentina, *N–Z*, giving the usual particulars of career, research activities and publications (Pp. 453–706. Montevideo: Unesco, Centro de Cooperacion Cientifica para America Latina, 1960).

Industrial Abstract, No. 47 (November 17, 1960), issued by the Technical Information Section, Federal Ministry of Commerce and Industry, Lagos, Nigeria, includes the abstracts classified according to the Universal Decimal Classification, indicated by its title. There are also a review of the fourth edition of the *Handbook of Commerce and Industry in Nigeria* and a list of industrial, scientific and commercial fairs, exhibitions and conferences, November 1960–February 1961 (Pp. ii + 26. Lagos: Federal Ministry of Commerce and Industry, 1960).

The Clay Minerals Group of the Mineralogical Society is holding a joint meeting with the British Society of Soil Science in the School of Agriculture, University of Cambridge, during April 6–7. The subject for discussion will be "Weathering". Further information can be obtained from Dr. R. M. S. Perrin, School of Agriculture, University of Cambridge.

The Advisory Committee on Arid Zone Research and the World Meteorological Organization are jointly organizing a symposium on "Changes of Climate" at the headquarters of the Food and Agriculture Organization, Rome, during October 2–7. The symposium is being arranged with special reference to Arid Zone Research. Further information can be obtained from W. Moller, Arid Zone Unit, Department of Natural Sciences, Unesco, Place de Fontenoy, Paris-7e.

A conference is being organized jointly by the Colour Group, the Institute of Physics and the Physical Society and the Inter-Society Color Council of America, to celebrate James Clerk Maxwell's demonstration of trichromatic colour reproduction at the Royal Institution in London. The conference will be held at the Imperial College of Science and Technology during May 16–18. Further information can be obtained from the Institute of Physics and the Physical Society, 1 Lowther Gardens, Prince Consort Road, London, S.W.7.

SCIENCE PLANNING, DEVELOPMENT AND CO-OPERATION IN THE COUNTRIES OF THE MIDDLE EAST AND NORTH AFRICA

A REGIONAL conference on the above topic was held under the auspices of Unesco and the United Arab Republic Science Council in the Senate Hall of the House of Representatives, Cairo, during December 19–23. The meeting was organized by the U.N. Science Co-operative Office of the Middle East (director, Mr. Jan Smid) and the United Arab Republic Science Council (secretary-general, Dr. A. F. Ismail). The meeting was attended by delegates from Iraq, Jordan, Lebanon, Morocco, Sudan, Tunisia, Turkey and the United Arab Republic. Three international scientific consultants were in attendance —Prof. J. Coulomb, directeur-général, Centre National de la Recherche Scientifique, Academician M. M. Dubinin, Academy of Science, U.S.S.R., and Prof. M. Stacey, Mason professor and head of the Chemistry Department, University of Birmingham.

The meeting was opened by an address of welcome from the Egyptian Central Minister of Education, His Excellency Kamal El Din Hussein, who discussed the need for co-operation in science and technology at regional and international level, and who emphasized the importance of such a conference for the future development of Middle Eastern and North African countries.

He was followed by Mr. Jan Smid, who spoke of the role of Unesco in the development of scientific research throughout the world. He outlined Unesco's efforts in trying to solve problems of economic planning in the less-advanced countries. He indicated the aims of the Conference as being of particular value in assisting the delegates to set up in their own countries some form of permanent centralized national body for co-ordination of scientific research aimed at better economic development of natural resources.

He emphasized the role of the international consultants. They were present to give their views on science administration based on their experience.

Each of the consultants then gave a short address on science planning and administration within his own country.

After an intermission, Dr. M. A. Haseeb of the Sudan was elected chairman of the conference and the elected vice-chairmen were delegates from Iraq and Turkey. Dr. A. F. Ismail of the United Arab Republic was elected recorder of the meeting. During the following two days delegates gave extensive reports on what was happening in science in their own countries. These may be summarized as follows:

Iraq. Delegates Dr. A. T. Al-Najim, director-general of education, Baghdad; Dr. F. Al Tai, dean of science, University of Baghdad.

The University of Baghdad was prominent in leading some research activities, and there were two research institutes in the country. Studies in agriculture and forestry were prominent and so were science and medicine. A number of research centres for industry, for example, for petroleum chemistry, were already planned, and schemes were in hand for atomic studies. There was a serious lack of trained scientists and technicians. Much thought was already being given to planning the national effort.

Jordan. Delegates Dr. S. Nashef, director of research, Ministry of Agriculture; Mr. A. Fouzi, under-secretary, Ministry of Public Works.

Jordan had no university, but there was a growing awareness of the importance of scientific research for the future development of the country. Various ministries had undertaken important research projects, for example, in agriculture, health, public works, etc. The Jordan Development Board, through its planning committee, was undertaking much research. Many industrial firms such as the Potash Co. were applying results of research. Many students were being trained abroad.

The country was beset with many difficulties. There was an urgent need for a central body for co-ordinating and financing research. There was a serious lack of scientists and trained technicians and a need for raising the status and salaries of those they had at present.

Lebanon. Delegates Mr. Joseph Naggar, president of the Central Scientific Commission for Lebanon; Dr. M. Attie, director, Industrial Research Institute.

There were three universities in Lebanon: (1) The Lebanon University, a recent foundation with quite well-equipped laboratories for research, especially in chemistry, physics and biology. (2) The American University with well-equipped science laboratories and with attached Schools of Engineering, Medicine, Agriculture and Economics. (3) The University of St. Joseph—a French University with good departments, but now lacking in funds.

There are a number of research institutes and also two good observatories. Scientific research in Lebanon lacks co-ordination, and urgently needs planning at the national level.

Morocco. Delegate Dr. Abdel L. Bennis, director, Agronomical Research Centre of Rabat.

There are a number of scientific research institutes in Morocco, for example, the Laboratory of Chemistry and Physics with divisions of hydrology, biological chemistry, hygiene, etc. There are also Laboratories of Parasitology, Entomology, Microbiology and General Biology. There are Departments of Botany, Chemistry, Geology (with an Institute of Geology) in what is termed the Faculty of Science of Morocco. An Agricultural Research Institute is under the care of the Ministry of Agriculture. There is a scientific institute, L'Institut Scientific Cherifeon, which was founded in 1921 with the object of co-ordinating science, and it has done valuable work in many disciplines and has given help with founding libraries, museums, etc.

There is a great need for a central science council for which the Government has now given approval. There was a grave shortage of scientists and technicians.

Sudan. Delegates Dr. M. Ali Haseeb, assistant director of research, Ministry of Health; Mr. A. A. El Karib, assistant director, Ministry of Animal Resources.

This country has 11 million people spread out over a million square miles. Its climate varies from desert to tropical and subtropical and it has rich natural resources of minerals and agricultural products. Cotton is the main export.

The University of Khartoum is well established, and is very active in many faculties such as Science, Agriculture, Engineering, Veterinary, Arts and Law. A good deal of research is applied for national needs. There are many institutes of the various ministries which carry out research, for example, Agriculture, Irrigation, Animal Resources, Tropical Medicine. The Government is sympathetic to potential developments through science. Many more scientists and technicians are needed in the country.

Tunisia. Delegates Mr. Hamza, head of the Faculty of Sciences, director of research on arid zone problems, University of Tunis; Mr. Talbi, professor, Faculty of Letters and Science, University of Tunis.

The country has some traditions in science, for the Pasteur Institute of Tunis was created many years ago. Researches in the natural sciences, etc., have been carried out in institutes under various ministries, for example, of Education and Agriculture. Connexions with France have been close, and results of researches have been published in French scientific journals. Now a University of Tunis has been created (March 1960) which, although needing 10 years to build, will cater for 15,000 students and will have powerful science faculties and research facilities. The general outlook for science collaboration in Tunisia is promising.

Turkey. Delegates Prof. H. N. Pamir, University of Istanbul; Prof. N. Serdaroglu, Technical University, Istanbul.

Scientific research is carried out in the Universities, in laboratories attached to ministries and at a few special institutes. There are six Universities—Istanbul, Ankara, d'Ego, Ataturk, the Technical University at Ankara and the Technical University of Istanbul. Between them most scientific disciplines are taught, and research facilities are good. Most specialists' subjects such as agriculture are studied in the Ministry Laboratories, of which there are eight. The special research institutes are medical, for example, for tuberculosis and cancer. Much thought is being given at present to the formation of a council for science, but many difficulties must be overcome.

United Arab Republic. Delegates Dr. A. R. Tourkey, director, National Research Centre, Cairo; Dr. M. M. Ahmed, vice-rector, University of Cairo; Dr. A. F. Ismail and Dr. M. K. Tolba, Science Council; and Dr. M. B. Ahmed, University of Cairo; Dr. Y. Khouri and Dr. S. Al Safadi, University of Damascus.

Comparatively speaking, the United Arab Republic is very well organized, and the powerful Science Council which co-ordinates science in Egypt and Syria has just published its third annual report together with a report to Unesco. Both of these make impressive reading. The five Universities—Cairo, Ain Shams (Cairo), Alexandria, Assiout and Damascus—are all being considerably expanded, especially on the research side. The building of the large science centre in Cairo is well advanced, and there are many other research institutes, for example, for atomic research.

The projects being attempted are largely aimed at developing natural resources and are making good progress. The United Arab Republic is already playing the part of a centre of scientific training for the region. Its own problems, however, are still very great: especially a need for more high-quality scientists to act as research leaders, for skilled technicians, for better information and library facilities, for expensive apparatus, etc.

In the lively discussions which followed, many important and not unfamiliar points emerged. It was clear that there is already a good foundation in most countries on which sound national and regional developments in science can be built. There is urgent need for firm and substantial government support, for fair treatment of scientists and for good co-operation through Unesco.

Finally, it was possible for the conference to pass the following Resolutions:

(1) That a central science council or a similar body should be established in each Member State with the aim of organizing, reinforcing and co-ordinating pure and applied scientific research as well as promoting research projects leading to the economic, social and intellectual development of the nation.

(2) That this council or body has public utility status and financial autonomy, and is directly attached to the office of the Prime Minister, having a limited membership including representatives of universities, interested ministries and scientific institutions as well as certain scientists and experts in industry and agriculture.

(3) That the Member States allocate to this council a sufficient budget which would enable it to accomplish its mission, and that total expenses for scientific research in the country are progressively increased in order to attain not less than 1 per cent of national income.

(4) That this central body should first make use of the existing research institutions by improving their possibilities and promote establishment of new laboratories in fields essential to national needs.

(5) That this council establishes the priority of various projects of applied research of national importance after preliminary study made by special committees formed by the council.

(6) That the council executes the adopted research projects either in its own laboratories or in co-operation with other competent scientific institutions.

(7) That a national scientific documentation centre be established in each Member State, with the aid of Unesco if necessary and on request, which will be attached to the council and charged with providing scientists with the necessary scientific information.

(8) That the Member States undertake adequate training of scientists necessary to the country by strengthening faculties, universities and institutions, and by establishing new ones as well as missions abroad.

(9) That the Member States define and establish the appropriate status of scientific workers together with provision of the material conditions corresponding to the highest scale existing in the country.

(10) That the Member States give attention to the training and improvement of material and moral conditions of auxiliary and technical personnel of laboratories.

(11) That the public and private resources grant housing and financial assistance to universities and institutions of scientific research to enable them to undertake the execution of their projects as is done at present in other countries.

It is recommended to the Member States that such grants are exempted from all taxes.

(12) That the council has a duty to promote regional and international co-operation, to elaborate the scientific parts of bilateral and international cultural agreements and to represent the country in international scientific non-governmental organizations.

(13) That the Member States grant sufficient means for the exchange of their professors and scientific workers and request Unesco to anticipate in its budget for 1963–64 a supplementary provision to accomplish this aim.

(14) That the Member States promote organization of national or regional scientific congresses and symposia as well as participation in international meetings.

(15) That the Member States support their scientific institutions in co-operation with similar establishments in other countries to solve problems of regional or international importance.

(16) That Unesco should continue to promote the organization of symposia and regional scientific meetings to discuss subjects of interest to various Member States.

(17) That all efforts and results reached by science should be used solely to help humanity and to improve the standard of living of the peoples.

(18) The conference thanks the Middle East Science Co-operation Office of Unesco, the Science Council of the United Arab Republic as well as the international consultants, and recommends that Unesco should organize within two years a similar conference to study the progress made in the field of scientific research in the Member States.

ELECTRICAL ENGINEERING IN THE ROYAL NAVY

AT almost the same time as he relinquished, by retirement, the post of director of electrical engineering at the Admiralty, Sir Hamish Maclaren assumed office as president of the Institution of Electrical Engineers. This is only the second occasion on which the chief electrical engineer of the Admiralty has occupied the presidential chair of the Institution. C. H. Wordingham, who became head of the Electrical Engineering Department of the Admiralty in 1903, was president of the Institution during 1916–18.

Very appropriately, in his presidential address delivered on October 6, Sir Hamish reviewed the development of electrical engineering in the Royal Navy. The electronic equipment and control gear of the submarine utilizing nuclear propulsion plant represents the culmination of some ninety years of development of the applications of electricity in naval vessels, the first being the electrical firing of guns. It was, however, nearly twenty years earlier that the Admiralty made its earliest uses of electrical signalling when in 1852 the twenty-two semaphore stations between Plymouth and Whitehall were replaced by an electric telegraph.

The first dynamo was installed in a warship in 1875 to provide power for a searchlight to be used against torpedo craft, and it is probably this early association with torpedoes that established the practice of placing the responsibility for all electrical equipment in ships on the torpedo officer. This practice remained in force until 1946 when the electrical branch was founded.

A combined ship lighting and searchlight installation was fitted in H.M.S. *Inflexible* in 1881, Swan incandescent lamps being connected in groups in series. By 1886 first-class ships were being equipped with three dynamos each supplying 200 amp. at 80 V.

The ring-main system of distribution at 22-V. direct current was introduced in the early years of the present century and remained the standard naval practice until after the end of the Second World War.

It was in 1896 that the remarkable pioneering experiments in wireless telegraph communication between ships were made by Captain H. B. Jackson. Communication was established between H.M.S.'s *Defiance* and *Scourge* over a range of 5,800 yards. In 1899 Marconi equipment with a range of up to seventy miles was installed in four ships.

Experience during action in the First World War focused attention on the impracticability of maintaining so-called watertight enclosures watertight, and means were devised for isolating, automatically, flooded equipment from the supply.

After the War the Admiralty reviewed the experience, to date, of electricity supply in warships and in particular considered the possible introduction of alternating current. Considerable importance, perhaps an undue amount, was attached to the ease and convenience of speed variation of the direct current motor and direct current was retained.

The application of electricity in fire control systems was greatly advanced during the First World War. Individual components such as plotting tables, data transmission and communication instruments had, however, been developed largely by individual specialist firms, giving rise to diversity of design and practice.

It was towards the end of the First World War that the gun director system was introduced in which a gyroscope was employed as a stable reference to ensure that the guns fired only when the ship was in a horizontal position during rolling. This was a prelude to the day when directors and gun mountings would themselves be stabilized.

Between the two World Wars action was taken towards achieving uniformity of practice in instrumentation. Much effort was directed towards the design of fire control computers, and, as the importance of air attack increased, to the special problems associated with the control of high-angle anti-aircraft guns. Of the work done at the Admiralty Research Laboratory during that period mention should be made of the plotting table which automatically records the ship's position on a chart, of the 'magslip', a data-transmission unit of much greater accuracy than any other available at that time, and of an electrically controlled oil-operated remote-power system for searchlights.

The outbreak of the Second World War produced immediately the problem of combating the novel and ingenious devices which had been developed by the enemy. The first was the magnetic mine. Real success in sweeping the magnetic mine was achieved with the development of the towed buoyant-cable sweep whereby a relatively large magnetic field is produced in the sea by circulating current pulses of several thousand amperes round the loop of buoyant cable.

The degaussing of ships, at first effected by a single-turn coil embracing the hull, was, after considerable research, shown to be practicable with multi-core cable fitted inside the hull.

H.M.S. *Belfast*, damaged by a magnetic mine explosion in October 1939, exhibited extensive damage to electrical equipment from underwater shock. The shock accelerations and displacements resulting from non-contact under-water explosions were of a much higher order than those against which the equipment had been designed, and an extensive programme of experiment and of redesign of electrical equipment and of shock-proof mountings was undertaken.

The loss of the *Ark Royal* in November 1941 brought to light further important factors in the vulnerability of the electrical systems of warships. A hit by a single torpedo had the disastrous result of flooding one boiler room and the main switchboard room. The remote control of circuit breakers from the main switchboard was based on a system which the flooding rendered completely inoperative and services could not be restored although power was available for some time. Even the power supply was dependent, as the generators were driven by steam turbines, on the ship's main boilers. The redesign of the control system introduced relay control, energized from independent local supplies, and auxiliary diesel generators were provided.

Electrical actuation in the training and elevation of anti-aircraft guns was initiated in 1939, utilizing the rotating amplifier machine known as the 'metadyne', controlled by a magslip-data transmission system to which reference has already been made.

The introduction of radar for range-finding produced automatic following of the radar aerial so that the aerial, the gun director and hence the gun automatically followed the target. Fully automatic following of the target was achieved by designing the radar to provide its own misalignment signal and feeding the necessary additional information from the fire control computer and the stabilizer.

The action information centre has become the most concentrated part of the electrical installation of a warship. It is the focal point for radar and asdic information, for external and internal communication, aircraft and weapon direction and for navigation.

Alternating current was adopted in 1948 for all ships larger than mine-sweepers. Simplicity, reliability and the necessity to save space and weight in the large and complex electrical systems required made the advantages of alternating current supply overwhelming.

A supply frequency of 60 c./s. was adopted for the 440 V. three-phase system. The higher frequency of 60 c./s., in place of the standard power frequency of 50 c./s., had the great merit of a substantial reduction in the size and weight of machines and a higher maximum speed of 1,800 revolutions per minute. There was the added advantage of a common standard with the U.S. Navy. There is, of course, the disadvantage of more complex equipment where continuous variation of speed is required. Automatic voltage regulation is required, and a standard type of automatic voltage regulator utilizing a magnetic amplifier has been developed which can be used with generators of 150-kW.–1,500-kW. capacity.

Nuclear propulsion has revolutionized the performance of the submarine, which becomes at last a true submarine rather than just a submersible vessel. The nuclear submarine must, however, still have a battery for starting up and emergency standby although of smaller size than that for the conventional submarine. Because of the possibility of prolonged submerged operation, some of the battery problems encountered with conventional submarines became of even greater importance. The evolution of hydrogen on open circuit can be reduced by the use of grid alloys having a very low antimony content. Antimony-free grids would at the same time eliminate the production of the highly toxic gas, antimony trihydride, or stibine.

The electrical installation of the nuclear submarine is in fact much like that of the surface vessel with the addition of an electric drive of small power for emergency use. As in the surface vessel, an increasing amount of electronic and servo equipment is required, and there is in addition the equipment for reactor control and instrumentation. It is in the submarine, because of the acute limitation of space, that semiconductor devices offer special promise in their future application.

J. Greig

POWDER METALLURGY

An international conference on "Powder Metallurgy", sponsored by the Metal Powder Industries Federation and the Metallurgical Society of the American Institute of Mining and Metallurgical Engineers, was held in New York during June 13–17. At sessions throughout the first three days, papers were presented by authors and briefly discussed. Visits to plants were made during the last two days. The meetings were well managed and held to a precise time schedule which allowed little time for informal discussion. The hall, with a seating capacity of about four hundred, was filled for every session.

Dr. P. Schwarzkopf, honorary chairman of the conference, opened it with a review of a half-century of modern powder metallurgy, which he considers to have developed since Coolidge's paper of 1910 on the powder metallurgy of tungsten. He regards earlier powder metallurgy, such as that in pre-history, that by the Incas, and the British and Russian work on platinum, as detached from the development of modern technology to which they made little contribution. Powder metallurgy was a forgotten art at the start of this century, although in 1898 Welsbach patented its use to make osmium filaments. Coolidge's tungsten filaments were the first really usable product of modern powder metallurgy. Cemented carbides manufactured by powder methods came in 1922.

Work on the nature of the sintering process appeared in 1922 when Sauerwald published his first paper on the subject, which he thought of as a recrystallization process. Important later work on the theory of sintering included Russian papers published in 1945 and the work of S. P. Slater and G. C. Kuczynski. In recent times the latter worker has dominated thinking in this field.

Several papers at this conference made it clear that work on the theory of sintering is once again coming to life. It is now appreciated that we must not seek one dominant sintering mechanism for all materials, or even for one material at all stages of the sintering process, or in all circumstances in which sintering takes place. Kuczynski (University of Notre Dame) emphasized this by stressing the convenience of considering separately the two extreme stages of sintering when seeking to explain what occurs: these are: (1) the formation of necks between particles at the start of sintering, and (2) the disappearance of isolated pores towards the end of the process. The first stage is now fairly well understood, but the second is more complex and difficult to cope with. Unbalanced forces in the necks lead to flow of material in a viscous or plastic manner. He has shown that, if x is the diameter of the neck between sintering particles, and a the diameter of the particles, x^n/a^m is a function of time and the values of n and m are characteristic of the sintering mechanism which is operating:

$n =$	2	3	5	7
$m = 1$	Viscous or plastic flow	Evaporation and condensation	—	—
2	—	—	Volume diffusion	—
3	—	—	—	Surface diffusion

Recent observations of the sintering of copper wires twisted together show that volume diffusion is in this case the main mechanism. Kingery has shown it to be evaporation and condensation in sodium chloride. In glass it is viscous flow. Sintering of nickel at 1,400° C. is due to volume diffusion and that of iron at 850° to surface diffusion. Kuczynski described a very pleasing experiment on the sintering of copper-8 per cent indium. Indium diffuses more rapidly than copper in this alloy and where its concentration rises an indium-rich δ-phase forms. This phase was found in the necks between sintering particles, an observation confirming that diffusion is a mechanism of the sintering in this case.

The importance of grain boundaries is not yet clear. A. L. Pranatis and L. L. Seigle examined the sintering of iron, copper and nickel and observed the radius of the neck between particles to be proportional to time whether boundaries were present or not. However, they found that reduction did not occur in the volume of isolated voids in nickel at 1,400° C. unless boundaries ran into them, whereas B. H. Alexander and R. W. Baluffi observed shrinkage of voids within metal single crystals.

A few years ago M. Eudier reported that additions of hydrogen chloride to the sintering atmosphere accelerated the sintering of iron powder and improved the properties of the product. More recently J. Vacek described the spectacular effect of a trace of nickel in causing tungsten to sinter at half the temperature required for pure metal. Considerable interest was shown in such activated sintering, the practical significance of which is clear. That the nature of the sintering atmosphere may determine which sintering mechanism dominates a particular case was illustrated by Kuczynski's observation that single crystal particles of alumina sinter by volume diffusion when heated in helium containing a little oxygen, but by viscous flow in dry hydrogen.

J. Wulff (Massachusetts Institute of Technology, Cambridge, Mass.) has studied the effect of nickel on the sintering of tungsten. He reminded the conference that this remarkable effect was known to von Bolton long ago, but has been overlooked until very recently. Wulff found that additions of nickel are increasingly beneficial so long as a monatomic layer over the tungsten particles is being built up, but not after sufficient metal for this is present. He attributes the effect to diffusion of tungsten through the nickel. F. Eisenkolb (Technische Hochschule, Dresden) found additions of about 2 per cent of calcium fluoride to iron or steel powder to improve its sintered strength, but that larger additions reduced this.

It would be very interesting to know what effect these additions have on the Kuczynski coefficients of the sintering metal.

Kuczynski recalled attention to phenomena when two metals sinter together. Thus when copper and nickel are in contact at 1,020° C. the copper particles shrink and pits form in them near junctions with nickel, while the nickel particles expand. This is explained by the more rapid diffusion of copper than of nickel.

G. A. Geach and A. A. Woolf (Associated Electrical Industries, England) have examined a number of pure crystalline organic materials of widely differing molecular shapes and found that these did not sinter at 0·8 of the absolute melting points, although they show creep deformation at lower temperatures. It is not clear whether this should be attributed to an absence of self-diffusion of large molecules or to the low surface energy of such materials. Although nylon sinters it is interesting that it does so more slowly if the threads are stretched so that the material becomes more crystalline. It was reported that in commercial practice amorphous precipitated nylon is found to sinter more effectively than ground material.

Developments were reported in many techniques of powder metallurgy. Although hydrostatic pressing of powder in a rubber sack was patented in 1913 much attention is now being given to this method. H. H. Hirsch and C. E. van Buren (General Electric Co.) reported that pressing vessels up to 20 in. in diameter and several feet long now exist, and that pressures up to 50 t.s.i. can be used, although it is now known that for each material there is a pressure above which little advantage is gained (30 t.s.i. for tungsten, 4·5 t.s.i. for alumina). Isostatic pressing to finished form in shaped moulds of rubber or reversible gel is extending, and may lead to economy when only moderate numbers of parts are required. Slip casting of metal powders in porous moulds is also being developed, and A. Poster (Sylvania Electric Products) described freezing the mould at 29° F. to remove the casting safely, and then drying it for two days in fuller's earth because rapid drying damages the surface. Hot extrusion of metal powder is particularly valuable when pyrophoric powders, or those involving a health hazard, are pressed while in a closed can. Developments in the direct rolling of metal powder to strip which were described included the use of heated rolls. Rolls of very large diameter are desirable for compacting powders, and an interesting technique described used two rings of large diameter of metal which were threaded about the 8-in. rolls of a smaller mill and operated as large rolls.

The use of an explosion to drive the ram of a mould pressing powders will give pressures of 10^6 p.s.i. for microseconds: R. A. Cooley (Propellex Division, Chromalloy Corp.) reported improved properties of products pressed in this way. A remarkable result claimed is nickel of density one per cent higher than the crystallographic value.

A large amount of work on dispersion-hardened materials was reported, and at present interest is greatest in materials based on aluminium, copper or nickel. It appears that the dispersed phase should be hard and that interfacial energy between it and the matrix should be low: thus thoria particles in titanium alloys have little effect unless the alloys wet the particles. Dispersed particles should be of irregular shape, not rounded, for greatest effect. F. V. Lenel and G. S. Ansell (Rensselaer Polytechnic) stated that the yield stress of these materials is determined by the stress which fractures a dispersed particle, and that it is inversely proportional to the square root of the spacing between particles.

SAP alloys (aluminium strengthened by dispersed oxide) are now known which can be rolled either hot or cold or which can be drawn and pressure-welded.

M. Eudier (Metallurgie Française des Poudres) reported that a controlled oxidation of nickel before sintering raises the density achieved, and he explained that the effect is due to filling of certain pores with oxide and later reduction of this without re-formation of cavities.

The powder metallurgy industry is expanding rapidly. The use of iron powder showed an upsurge during 1954–55, and in 1960 was three times that in 1956. Application of powder methods to beryllium is now important, and the plant to handle a large amount exists. Consolidation of powder in cans by quick blow-forging is important and disks 12 in. in diameter by 1·5 in. thick are made in this way. Beryllium foam made from fine powder, and the production by hot pressing of metal beryllide parts which have high thermal conductivity, are novel developments. Very large pieces can now be manufactured by powder methods: F. Emley (Westinghouse Electric Corp.) noted equipment for hot-pressing beryllium billets up to 60-in. diameter, and the production of molybdenum billets 40 in. × 3·5 in. × 3·5 in.

G. A. Geach

SOIL FAUNA IN RELATION TO SOIL FORMATION AND FERTILITY

THE study of soils has for long been the province of pedologists, chemists and microbiologists, and, apart from isolated instances, it has only been during the past fifteen years or so that the subject has attracted attention from zoologists in Britain. During the 1955 Easter School held by the School of Agriculture, University of Nottingham, many soil zoologists from Europe as well as Britain were brought together. This was succeeded during 1958 by a three-day international colloquium held at Rothamsted Experimental Station and devoted to "Progress in Soil Zoology". These have given new stimulus to the subject, and the recent symposium on "Soil Fauna in Relation to Soil Formation and Fertility", held by the Association of Applied Biologists, indicates the still growing interest which soil zoology is arousing. This symposium was held on October 14 at the British Museum (Natural History), where the new lecture theatre is eminently suitable for such meetings.

The theme was introduced and summarized by G. V. Jacks, director of the Commonwealth Bureau of Soils. He started by giving numerical evidence of the increasing number of publications on soil zoology appearing in *Soils and Fertilizers*: during 1937–40 there were three, during 1953–56 thirty, and during 1956–59 ninety. The part played by biological processes in determining soil fertility was then put in perspective. Under natural conditions soil animals are complementary to plants and necessary for operating the cycle of nutrients effectively. They are important factors in breaking down plant litter, mixing it with the soil and creating the porous texture characteristic of good soils.

The first paper was given by Dr. D. A. Osmond and Mr. P. Bullock, of the Soil Survey of England and Wales, on "Soil Fauna in Relation to Pedology". The condition of a soil is the result of many factors of which the fauna is but one. The influence of animals is difficult to assess in arable land, but may be very great in grassland and forest soils. Their effects range from the burrowing of mammals to the incorporation of quantities of plant material into the soil by the mesofauna. Dr. Osmond then described different soil-types with the aid of photographs of thin sections. Peat represents one end of the scale where plant matter is virtually unattacked by the fauna, and with increasing faunal influence one passes through moder and mull soils, finally culminating in the vermisols possibly found in parts of north England which are the result of intense earthworm activity.

The mechanics of earthworm influence were pursued by Dr. F. Raw, from Rothamsted Experimental Station, by a consideration of leaf burial in apple orchards. Comparisons were made between sprayed and unsprayed orchards, some under arable cultivation and others under grass. For sampling earthworm populations dilute formalin was found more efficient than permanganate, and in the seven orchards investigated six dominant species were found. Of these, pot experiments showed that only *Lumbricus terrestris* was responsible for pulling leaves into its burrows. Quantitative studies showed, moreover, a close relationship in the grass orchards between weight of *Lumbricus terrestris* populations and the percentage disappearance of leaf litter from cages. The rate of disappearance was inversely proportional to the amount of surface vegetation, being greatest in the arable orchards.

In grass orchards which had been sprayed with a copper fungicide for many years, the earthworm fauna was reduced to a single species living in the surface litter. Here leaf decomposition was very slow and the soil profile had typical mor characteristics.

Some aspects of work being carried out at the University College of North Wales, Bangor, were described by J. Hobart and A. J. Hayes. The former gave a paper on the distributional patterns of soil mites in pure and mixed stands of conifers. In the apparently uniform habitat of a Douglas fir plantation (*Pseudotsuga taxifolia* Britt.), only eight out of thirty-two common species of mites (adults) did not show a significantly aggregated distribution. Samples were taken along radiating transects from trees, and several species of mites were found to have very definite and different preferences for particular radial distances. These may be due to differences in the thickness of the litter layer and in moisture content, and are evidence of very sensitive discrimination.

In mixed plantations, again, distinct populations were found in relation to spruce (*Picea* sp.), Scots pine (*Pinus sylvestris* L.), larch (*Larix* sp.) and silver fir (*Abies alba* Mill.). The numbers under spruce were generally lowest, and a few species showed definite preferences for larch or silver fir.

Hayes described some work on feeding behaviour of Oribatid mites using mainly *Hoploderma magnum*. Colonies were kept at constant temperature maintained at three different humidities and fed on weighed quantities of needles from three species of conifer. These needles were graded into three categories of age and condition. After 72 hr. the remains were re-weighed and the number of fæcal pellets counted. Measurements were made on eight replicates of fifty individuals for each treatment. Production of fæcal pellets for all three species appeared to be greatest for fallen but still entire needles and least for fresh green needles. Analysis of variance showed, however, that few of the differences between humidities, tree species or conditions of needles were significant. Preliminary results indicated that there was no correlation between loss in dry weight and the number of fæcal pellets produced, and loss in dry weight of needles did not appear to give a satisfactory index of feeding.

As part of the concerted work on woodland soils by the Nature Conservancy at Merlewood Research Station, K. L. Bocock described some work concerned specifically with the comparative rates of disappearance of oak (*Quercus petraea* Liebl.) and ash (*Fraxinus excelsior* L.) leaves on contrasting sites. Weighed quantities of leaves were placed on the ground in nylon hair-nets and sampled periodically over 14 months. As a result of earthworm activity ash leaves on a mull soil disappeared most rapidly, with loss in nitrogen following closely the trends of loss in dry weight. On a moder soil the loss of nitrogen was relatively less rapid. Oak leaves on mull showed an actual increase in nitrogen content; possible explanations were the presence of nitrogen-fixing micro-organisms, absorption of nitrogen compounds from the rain or contamination with slime and droppings from soil animals.

Feeding experiments with *Glomeris* (Diplopoda) on hazel leaves (*Corylus avellana* L.) showed that the amount of water-soluble nitrogen was larger in the fæces than in the food, largely due to an increased ammonia content.

The symposium ended with a synoptic point of view of invertebrate metabolism and its effect on soil fertility. With the aid of diagrams, A. Macfadyen depicted the main paths of energy-flow in a typical grassland soil as a result of the direct metabolic effects of the fauna. Calories entering the system from sunlight pass through various levels (corresponding to successive links in food chains) at each of which some energy is dissipated in respiration. The proportions received and passed on by different groups of organisms and, in the case of agricultural exploitation of the grassland, the amount finally available to man were calculated.

Indirectly the fauna has catalytic effects on other organisms which are probably much more important: the feeding of animals on fungi eliminates senescent growths, effects the dispersal of spores and overcomes 'stasis', and their fæces provide food sources for other organisms. All such activities increase the rate of energy-flow which ultimately determines the soil fertility.

B. N. K. DAVIS

AGRICULTURAL HISTORY

THE joint winter conference of the Agricultural History Society and the Association of Agriculture was held on December 3, at the Institute of Education, University of London, under the chairmanship of Sir Keith Murray. Before lunch, Mr. Michael Havinden, research assistant, Museum of English Rural Life, read a paper on "Agricultural Progress in Open Field Oxfordshire". Modern research has suggested that the open field system was more flexible than was formerly believed. Mr. Havinden's researches among college documents and seventeenth-century Oxfordshire farmers' inventories has confirmed this still further. He is convinced that the introduction of improved rotations by agreement among the tenants or imposed by manorial lords was a major factor in delaying enclosure in Oxfordshire and possibly elsewhere. The improvement was mainly towards the introduction of fodder crops, the growing of more peas and beans and vetches, and the new legumes clover, sainfoin and lucern as well as some rye grass. In places a group of strips was enclosed by agreement and these crops were grown. He described the mechanism of this process very definitely. In other places fodder was grown and consumed by hitching the grazing animal on the strip. It has, of course, for long been known that sainfoin was grown on the chalk escarpment in the seventeenth century, but only vaguely. Mr. Havinden's researches have revealed places where this and other fodder crops were actually grown in the open fields, and he has thus made specific what was formerly only a general impression derived from remarks in the didactic treatises and propagandist literature of the time.

In the afternoon Mr. John Saville, senior lecturer in economic history, University of Hull, discussed "Public Opinion and Agricultural Depression, 1880–1900". He showed how the current difficulties of the landed interest had led reformers to demand the restoration of a peasant proprietary without any very clear perception of the methods by which the plan could be put into effect. Despite some ameliorative legislation, giving the farm worker the vote, the setting up of parish councils, some small-holding acts, etc., the ideas were in the main quite ineffective. These measures had been supported by liberal reformers in the first place, but afterwards received the backing of the great landlords, Henry Chaplin for example. In spite of that, somewhat less than 1,000 acres of small holdings were created before 1914.

The programme was closed by the showing of a film made by Imperial Chemical Industries in 1942, "The Harvest Shall Come", which is a moving history of the wretched conditions of employment in agriculture before 1914, the comparative prosperity of the workers during the two Wars, and their submersion in poverty in the interval between them, conditions that will never again be imposed.

PROGRESS IN BRITISH BROADCASTING

THE annual report and statement of accounts of the British Broadcasting Corporation for the year ended March 31, 1960, comprises a general review of the year, the reports of the Broadcasting Councils for Scotland and for Wales, chapters on programme output, regional broadcasting, audience research, external broadcasting, engineering, staff and contractual matters, publications and finance, together with a series of appendixes and tables[*]. Matter of scientific interest is contained mainly in the general review and in that on engineering. The general review notes that plans are ready for substantial advances in sound broadcasting, which shows every sign of maintaining a hold on the public in its own right and as a service complementary to that of television. In the overseas and international spheres, further developments and possibilities lie ahead for the Corporation, especially in the field of television, and, generally, the Corporation considers that it has gone a considerable way to map out the courses that it should follow and the programme of development that will be needed during the next five to ten years.

The Corporation welcomes the Government's decision that during 1961–62 the full net proceeds of the licence revenue, apart from the £1 excise duty, should be devoted to broadcasting. However, the retention by the Treasury of 5 per cent of this revenue in the current financial year has necessitated the slowing down or postponement beyond 1960–62 of some projects which the Corporation regards as highly desirable, and which it wishes to complete at the earliest possible date. During the year the number of householders with combined licences for sound and television rose from 9,255,422 to 10,469,753, and that of those with licences for sound only decreased from 5,480,991 to 4,535,258. About 97 per cent of the population of the United Kingdom are now within range of the VHF sound broadcasting network, and it is estimated that rather more than one household in five has a VHF receiver. In sound, as in television, the Corporation is planning to provide or improve coverage in certain difficult areas by building low-power satellite stations, and schemes have also been prepared both for a further extension of coverage and also for introducing a system of local broadcasting on VHF. Audience research indicated that adult viewers with a choice of BBC and ITV were devoting some 37 per cent of their evening viewing time to BBC programmes in the last quarter of the year, compared with 32 per cent in the first quarter.

Experience gained during the past eleven years suggests that the restriction on broadcasting imposed by Section 63 of the Representation of the People Act, 1949, is now out of date, and the report expresses the hope that action will be taken to amend the Act accordingly. In science broadcasting, Prof. Medawar's Reith Lectures took an outstanding place, and upwards of 150 leading scientists took part in science programmes in sound radio during the year, while in television "Eye on Research", in which scientists in many fields demonstrate their equipment and research techniques and explain the work that they are doing, was the main continuing contribution. From time to time, however, the full resources of television are deployed on some major scientific theme, but the series of televised scientific demonstrations given by Sir Lawrence Bragg in the main lecture theatre of the Royal Institution were also highly appreciated by viewers. Preparations went forward throughout the year for the increase in output of the school television service to take place in the 1960–61 school year, and the scope of the service will also be extended to cover some of the special needs of primary schools and of the sixth forms of grammar schools.

Referring to regional broadcasting, the report expresses the view that if full advantage is taken of the opportunities for development, in adjustment to the changing needs of the time, regional activities in sound will form an enduring part of the overall pattern of the Corporation's broadcasting; but, while sharing fully the desire of the Broadcasting Council for Wales for an allocation of frequencies which would permit a separate television transmitter for South Wales, the Corporation has been unable to take the necessary steps because of the insufficiency of channels available. At present the Corporation's television service covers 98·8 per cent of the population of the United Kingdom, but the technical problems in bringing this to 100 per cent are formidable and are discussed in some detail in a special section of the review dealing with future tasks. The first of these is seen as the extension and improvement of transmitter coverage in areas where reception, whether of sound or television programmes, is unsatisfactory or non-existent. For television the only frequencies that could be readily applied for this purpose are the as yet uncommitted channels in Band III, and if the Government decides that these should be used for an additional television service, the Corporation wishes to provide the additional service. Apart from giving viewers a genuine alternative service, this second service would enable the Corporation to increase the number of serious, cultural and informative programmes, to cater more fully for regional needs, to extend educational broadcasts, and to provide more opportunity for experimental programmes. The Corporation believes that a move into Bands IV and V, which is an essential condition for a change in television standards, can be made successfully only if the public has the incentive of one or more new programmes in these bands. Given the necessary frequencies and finance, the Corporation would be prepared to start a new service in these bands and also if the Government so decided to start a service of colour television in Bands I or III, taking advantage of the experience it has already acquired and of the many experimental transmissions it has made in colour using the present 405-line standard. The Corporation claims that it could start a second television service in Band IV if it were granted the full proceeds of the present £4 licence, and that full development of the Corporation's services in sound and television, including a new television service in Bands IV and V and of colour, would call for a combined licence at about £5.

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[*] Annual Report and Accounts of the British Broadcasting Corporation, 1959–60. Pp. 175+8 plates. (Cmnd. 1174.) (London: H.M. Stationery Office, 1960.) 8*s.* net.

Under engineering research and development, the report notes that a comprehensive report has been prepared for publication on a series of experimental transmissions in Band V which included a comparison of the existing British 405-line standard and the Continental standard of 625-lines. The regular experimental stereophonic transmissions, commenced in 1958, have been continued, and the Corporation is investigating possible systems of broadcasting both stereophonic channels from a single VHF transmitter in such a way that monophonic reception is not impaired. Research continued into various aspects of videotape recording as well as investigation of the propagation properties of the ionosphere, for purposes of day-to-day operation of the Corporation's short-wave services, and considerable effort has been directed to the design of equipment using transistors instead of valves, although in broadcasting, and particularly in television, it is difficult to obtain the same high standard of performance from transistors as can be obtained from valves. Nevertheless, in almost all new portable or mobile audio-frequency equipment, and also in fixed equipment designed to operate independently of the public mains supply without large and costly batteries, it is possible and advantageous to use transistors.

While there is a marked potential audience for the Corporation's external broadcasts, to which the development of transistor receivers, cheap and easy to run, has made an important contribution, especially for medium-wave transmissions, Communist China has now displaced the BBC as the third largest external broadcaster. The services have been extended in certain directions, but rising costs and the stringent limitation of grants by the Government have offset the expansions by reductions elsewhere, and the report stresses the challenge which faces external broadcasting from the United Kingdom. In meeting it, the Corporation's international reputation for reliability and independence, its long experience and its established position as a source of programmes regularly heard and re-broadcast throughout the world are solid advantages, but they require ample technical support, particularly by way of higher-powered transmitting stations in the United Kingdom and more relay stations overseas. When this is forthcoming, the BBC will be able to take greater advantage of the many opportunities for presenting Britain to the world through this medium.

SCIENTIFIC RESEARCH IN NEW ZEALAND

THE report of the Department of Scientific and Industrial Research, New Zealand, for the year ended March 31, 1960, shows gross expenditure of £1,812,000, and staff at April 1, 1960, totalling 1,121, including 401 professional, compared with 1,067 and 390, respectively, in the previous year*. It includes the Minister's statement, the report of the Council for Scientific and Industrial Research, the Secretary's report, notes on the work of the several branches and divisions as well as of the incorporated research associations and on grant-aided research in other institutions, and also lists of publications. The second annual report of the Ross Dependency Research Committee is appended, to which also is attached a list of publications. A survey of the work of the Department made by the Council during the past two years led to the conclusion that in most branches the balance was held between *ad hoc* and long-term basic research, though in a few units basic research could with advantage be strengthened. Over the past decade the number of scientific staff employed by the Department has remained virtually static and there is virtually no scope for readjustments to meet the demands of new developments in nuclear science, oceanography, geothermal or Antarctic activities. Limitations of staff and finance debar the Department from exploiting fully the scientific opportunities to assist primary and manufacturing industry, and the Council estimates that full implementation of the present programme of work requires an increase of 40–50 staff per annum and of expenditure from £1,812,000 in 1959–60 to £2,090,000 in 1960–61, £2,133,000 in 1962–63 and £2,265,000 in 1964–65, assuming that costs and salary scales remain at the 1959 level. Provision of £200,000 per annum for buildings over the next five years is also recommended, apart from the cost of some major items of equipment such as a small nuclear reactor and electronic computer. Establishment of a National Research Organization as an independent organization responsible for the main research functions of Government is recommended as likely to lead to greater efficiency, better co-ordination in existing research programmes and greater flexibility in meeting future needs, and finally the urgent need for a thorough revision of professional salaries is emphasized.

Since 1949–50 the expenditure of the Department has risen from £959,000 to £1,658,000 in 1958–59, and was only 0·146 per cent of the gross national product, compared with 0·173 per cent, and the professional staff numbered 383 compared with 363. Research grants to universities, research institutions, etc., in 1959–60 totalled £172,272, but the only branches of the Department in which expenditure exceeded £100,000 were the Dominion Laboratory (£194,180), Dominion Physical Laboratory (£199,203), Geological Survey (£102,750), Geophysics Division (£111,206), Grasslands Division (£108,078) and Soil Bureau (£105,512).

The Secretary's report refers briefly to some of the research work of the year. An extensive programme of tests on the durability of paints showed that poor workmanship and inferior quality of paint were the prime causes of poor durability, with excessive weathering of priming coats and the dip-treatment methods of timber preservation as contributory factors. More careful attention to surface preparation is required when using synthetic finishes. Use of sawdust or shavings from arsenic-treated timber in smoke curing has been shown to constitute a toxicity hazard through deposition of arsenic on the food. The analysis of steel and reinforced-concrete frame structures is being continued, and the use of an analogue computer offers a big advance on the traditional technique of applying a static seismic coefficient. The computer is being modified to allow for multi-storey yield.

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* New Zealand. Report of the Department of Scientific and Industrial Research for the year ended 31 March, 1960. Pp. 128. (H. 34.) (Wellington: Government Printer, 1960.) 4s. 6d.

A major addition to the equipment used in the auroral programme has been the auroral radar set up at Arrival Heights close to Scott Base, and the unmanned automatic observatory operating for a year near Becks in Central Otago has demonstrated the superiority of conditions there for all aspects of the optical work. The Geophysical Observatory has made a special investigation of the New Zealand ionospheric data to meet requests made by the Post and Telegraph Department, and a detailed magnetic survey was made on the aircraft compass-swinging site at Wellington Airport; this was to ensure that the area was free from magnetic effects due to buried ferro-magnetic materials that might affect aircraft compasses. Considerable attention has been directed to modern map-preparation techniques and procedures in cartography, and a major activity of the Antarctic programme has been the continuation of the geological and topographical survey of the Ross Dependency begun by parties from the trans-Antarctic Expedition. A major step forward in pollen research in New Zealand will be the publication this year of a *Geological Survey Palaeontological Bulletin* recording the important results of a new phase of the work—the successful extraction of pollen from marine sedimentary rocks.

In the past year the New Zealand benthic survey has concentrated on the west coast of the North Island and the western approaches to Cook Strait, and the survey is also building up collections of marine bottom fauna from which the common New Zealand species, and many of the rarer ones can be identified and described. Work on the design of a gas-pressure alarm system in telephone cables led to a method of calculating the required shapes of the pressure waves for a cable of given permeability to the gas for any size of puncture. At the Dominion Physical Laboratory the internodal cell of the alga *Nitella* is being used for an investigation of cell wall growth. Work has been carried out on the growth of *Pithomyces chartarum* on various synthetic media, and on the influence of different factors on the production of spores, while the fatty acid composition of this and related species has been determined and a systematic investigation made of the anti-fungal substances in sweet vernal and red clover.

Much of the development programme at the Keikohe sub-station of the Grasslands Division has been concerned with the typical heavy clay 'gumland', which is very acid, extremely low in plant foods and has a typical 'gumland' cover of stunted manuka and rushes. Heavy dressings of lime, phosphate and potash at the time of sowing gave spectacular results. Work on the South Island tussock grasslands has revealed two undescribed and widespread grassland associations of botanical and economic importance. Two high-yielding varieties of potato have been bred which appear to combine resistance to wind and drought with resistance to late blight, and use of systemic insecticides has opened up possibilities of producing better seed potatoes in areas such as Lincoln, where aphid numbers are high for only short periods. By use of stickers and cultures with a high bacterial count, nodule bacteria have been held on lucerne seed in a viable condition for at least four weeks, while work on scale insects on apples indicates that shelter belts of *Pinus radiata* are the cause of the trouble. Factors governing the need for potash top-dressing on New Zealand soils are also being studied as well as pruning systems for apples, the storage of apples in controlled atmospheres, the control of *Verticillium* wilt of tobacco and the general ecology of rabbits, while a survey at Lake Monk, Southern Fiordland, has contributed towards the understanding of the relations which have arisen between introduced animals and native forests.

THE DELHI ENGINEERING COLLEGE TRUST

At a meeting of the sponsors of the Delhi Engineering College Trust, held at Bowater House on November 16, the chairman, Sir William McFadzean, president of the Federation of British Industries, reviewed progress in the setting up of the College, in which H.M. Government, the Government of India and British industry are working together with the aim of establishing an outstanding institution for technical education.

Since January 1959, when Prince Philip laid the foundation stone of the College at Delhi, the Government of India has completed the administrative organization and has set up a governing body of which the chairman is Prof. M. S. Thacker, Permanent Secretary of the Indian Ministry of Scientific Research and Cultural Affairs. All preparations are in hand for the opening of the College during July 1961, on the magnificent Haus Kas site near Delhi.

The United Kingdom Government has undertaken to provide ten British professors for the College, who will advise the Indian professorial staff during the first five years. Four professors are already at Delhi, and three more are to be appointed this year.

Sir William also reported the success of the appeal to British industry for subscriptions to provide equipment for the College. The target figure of a quarter of a million pounds had been exceeded, thanks to 115 companies which had contributed over the past year. Over the next three years, as the buildings of the College are completed, the equipment for the successive stages of the five-year course is to be purchased on behalf of the Trust by the Crown Agents for Oversea Governments and Administrations.

Sir William then outlined the desirability, after the College was established, of offering continuing academic and practical support for it from the United Kingdom Government, British industry, and British universities and colleges. This was a matter to which the sponsors would be invited to give further attention when the time was ripe. In the meantime, the progress that was being made in all preparations for the opening of the College held out good promise for its future as a centre of advanced education in engineering in India.

Sir Eric Coates, chairman of the Executive Committee of the Trust, described how the most modern equipment for the College was being selected, in accordance with the advice of an academic working party under the chairmanship of Prof. S. R. Sparkes, of the Imperial College of Science and Technology; and how other aspects of the project were developing in accordance with the programme of the Indian Government.

Sir Willis Jackson, as chairman of the Technical Sub-Committee of the Trust, referred to the difficulties at the present time of finding the right calibre of man for professorial posts. He emphasized the need for further help by British industry, perhaps by endowing chairs at the College, and also by facilitating the secondment of experts in specialist fields of technology for short periods to assist in the conduct of postgraduate courses and in stimulating research.

Sir Algernon Rombold, Deputy Under-Secretary of State, Commonwealth Relations Office, said that satisfaction at the response to the sponsors' appeal would be widely shared, and that the project was an outstanding example of co-operation between the Governments of India and the United Kingdom and British industry. He endorsed the hope that a recognized system of secondment by industry and universities would be developed to meet the growing need for experts for overseas service.

Sir Alexander Fleck said that the vigour of the work of the Trust's officers showed that the special problems presented by any tripartite project of this nature were being solved in a satisfactory way. He believed that the continued support of British industry would be readily forthcoming for the project on the lines which Sir William McFadzean had sketched out. This would be true both in the immediate future and over the years as the project developed.

Among the other sponsors present were Sir Hugh Beaver, Sir Leslie Gamage, Mr. S. E. Goodall, Sir Kenneth Hague, Sir Harry Jephcott, Prof. Sutton Pippard and Mr. Whitney Straight. Other sponsors who were unable to attend were represented.

THE ROBERTSON CREEK SPAWNING CHANNEL

MUCH of a recent issue of the *Canadian Fish Culturist* (No. 27, 1960) is given up to accounts by K. C. Lucas and D. Mackinnon of a man-made spawning channel which has just been built by the Fish Culture Development Branch of the Department of Fisheries of Canada in Robertson Creek. This is a secondary outlet of Great Central Lake, which lies near Port Alberni on Vancouver Island, British Columbia.

The design of this new artificial spawning channel is based on the experience gained in the construction and operation of an artificial spawning channel on Jones Creek, near Hope, British Columbia, which was made in 1954. The Jones Creek channel was constructed to save the salmon stocks in a stream threatened by the diversion of the flow because of hydro-electric development, but the Robertson Creek channel is the first such channel to be built primarily as a fisheries development project. Robertson Creek is an exceptionally favourable area for such a project, because works for the complete control of flows already existed, no provision for flood-flows was necessary, silting of the spawning beds by the incoming water seemed unlikely, the temperature of the stream remained moderate and the area was readily accessible.

The total length of Robertson Creek is 4,200 ft., and 2,550 ft. have been developed as a spawning channel. The gradient of the natural stream bed was greater than that needed for the spawning channel, so four drop-structures have been provided to localize the unwanted drop. Near the head of the spawning channel and a short distance below Great Central Lake is a downstream migrant diversion structure, which allows migrants passing out of Great Central Lake to be diverted into a flume which runs alongside the spawning channel to enter the latter just below drop structure 3. If it were found necessary in the future, this diversion flume could be extended downstream so as to bypass the lower spawning area. The first three drop-structures downstream from the diversion structure are similar in design and are relatively simple structures. Inclined plane traps are provided at each so that downstream migrants can be counted, and each incorporates a pool and weir-type fishway to provide for the upstream movements of adults if desired The lowermost drop-structure (4) is larger and more complex, because it incorporates a fish holding and collection system, and there is an observation chamber built into the side of the fishway.

A number of rearing ponds have been built at one side of the channel near its upper end, and these have been specially designed to allow maximum flexibility. Another additional structure is of particular interest; this is an experimental test-flume, approximately 150 ft. long and 30 ft. wide, which lies in the channel a short distance below drop structure 3. Plans were made to construct this flume before it was decided to make the channel, and the Fisheries Research Board of Canada has contributed to its cost. This flume will be used for tests of fry and smolt guiding devices and for fundamental work on the physiology of adult salmon.

Previous work on the survival of Pacific salmon eggs and alevins has shown that the percentage survival varies widely, but is usually low (less than 10 per cent). The results of the first transplant of pink salmon eggs in the Robertson Creek spawning channel are therefore particularly encouraging More than one and a half million pink salmon were planted, at the eyed stage, during the latter half of November and the first days of December 1959. The first fry was trapped on March 15, 1960, but nearly all the fry migrated during the first three weeks of April and, when the last batch recorded migrated, during the night of April 21–22, the total represented roughly 95 per cent of the eyed eggs planted.

This artificial spawning channel is clearly a very important site for experimental investigations of many aspects of the biology of various stages in the life-history of Pacific salmon. Judged as a piece of experimental equipment, it is not cheap. To date, more than £81,000 has been spent on the channel. the experimental flume and the small field camp which has been built near the lower end of the channel It is becoming increasingly clear, however, that many problems of salmon biology cannot be solved unless adequate experimental facilities can be provided. and there is good reason to suppose, therefore, that this outlay will prove to be money well spent.

AN ATOMIC BEAM SPECTROPHOTOMETER

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THIS article describes an optical spectrometer of extremely high resolution which has been developed in these Laboratories during the past two years. It is similar in principle to recently described X-ray spectrometers based on the Mossbauer effect[1], but differs in that it is concerned with wave-lengths in (and near) the visible region of the spectrum.

The present instrument is based on the principle of scanning the spectral line under study by measuring the variation of intensity of transmitted light as the absorbing line is made to traverse the width of the emission line under investigation. Extremely narrow spectral lines of nearly natural line-width are obtained by using a highly collimated atomic beam[2,3] emitting, absorbing or scattering light at right angles (or nearly so) to the direction of motion of the atoms, thus reducing Doppler broadening.

This narrow spectral line can then be used (by the Zeeman, Doppler, Stark or modulation effects[4]) to scan the spectrum under study, and, from the variation of transmission (or resonant scattering) versus displacement, the wave-length of the centroid of the spectrum and its profile can be deduced.

The range of wave-lengths which can be scanned is restricted to the immediate vicinity of the resonance lines of the atoms composing the beam, although it may be possible to examine other than resonance lines by populating excited levels of atoms by resonance radiation or electron bombardment. In practice the Zeeman scan can cover a range of the order of ± 0·1 Å. for magnetic fields of 10,000 gauss. The range is usually very much smaller for scans relying on the Doppler shift, the Stark splitting or the modulation effect.

The resolution of the method is determined by the width of the spectral line used. If the resolution R is defined in terms of a criterion analogous to the Rayleigh criterion[4] we have $R = \lambda/\Delta\lambda$, where λ is the wave-length and $\Delta\lambda$ is the wave-length change of a perfectly monochromatic incident light-beam which will result in a 19 per cent change in absorption. Then the resolution R can reach 10^8 or more by collimating the atomic beam sufficiently to reduce the Doppler broadening, and it is ultimately limited by the natural line-width to a value $R = \lambda/\Delta\lambda = 4\cdot 1\ c/\lambda\gamma$ using the Lorentzian line shape, where c is the velocity of light and γ is the total transition probability per unit time[5]. Thus for mercury, ideally for a single even isotope of mercury, for example, mercury-198, we would expect[6] $R_{\text{max.}} = 5 \times 10^8$ for the 2536·5 Å. spectral line.

Two sealed glass atomic beam units (Fig. 1) were constructed to demonstrate the high resolution attainable with the above method. Because of its convenience, sodium was used as a working substance although its D lines have a complex structure. A low pressure was maintained by a small titanium pump[7]. An electric oven was used to heat the sodium to 300° C. and two glass slits of dimensions 2 × 18 mm. spaced 10 cm. apart collimated the atomic beam, which entered an observation chamber and condensed on a surface cooled with liquid nitrogen. To avoid appreciable Zeeman splitting of the spectral lines by stray magnetic fields in the vicinity of the observation chamber, the pumping magnets were removed during measurements. A pressure of 10^{-6} mm. mercury or less could be maintained for several hours without the operation of the pump by using sodium purified by multiple distillations, and baking the glass unit for many hours before sealing off.

Two such units were arranged as shown in Fig. 2. The sodium vapour lamp L illuminated the atomic beam E of sodium (the emitter) shown in plan. A second atomic beam unit A, with the atoms moving parallel to those of the emitter E, could be used as a resonant absorber. Of the light scattered at right angles to the direction of the atomic beam E, a beam subtending a very small solid angle served as a source of nearly monochromatic light of known line profile. The shape of this spectral line was examined by measuring the intensity of the light transmitted with the photomultiplier $D1$, as the absorber spectral line was moved across the emission line. The outputs of photomultiplier $D1$ and comparison photomultiplier $D2$, looking at the emitter only, were amplified in a difference amplifier and recorded on an XY recorder.

In the first experiment the practicability of Zeeman scanning was investigated by placing the absorber A in a magnetic field generated by a set of Helmholtz coils.

Light resonantly scattered by the emitter E passed through the absorber A parallel to the magnetic field

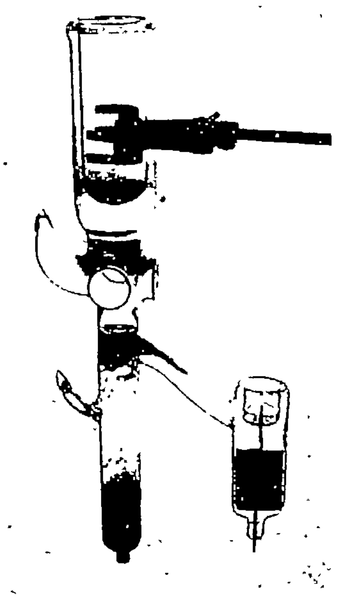

Fig. 1. A sodium atomic beam unit

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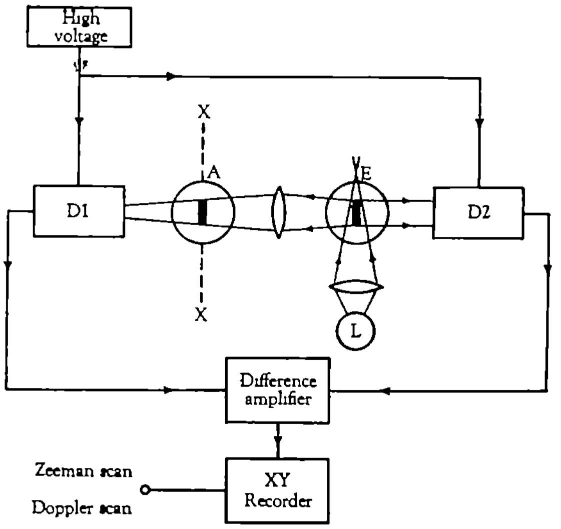

Fig. 2. Arrangement of apparatus for demonstration of high resolution of the method

and was detected by the magnetically screened photomultiplier $D1$ and recorded by the XY recorder as a function of the magnetic field by driving the X-axis with a voltage signal derived from the current through the Helmholtz coils. Electron-spin resonance in a small sample of $\alpha\alpha'$diphenyl picryl hydrazyl placed in a position normally occupied by the atomic beam was used to calibrate the linear magnetic field scale. Fig. 3 shows a typical Zeeman scan having a full width at half height of 67 gauss. This value is only slightly larger than the value calculated on the assumption that each hyperfine structure component of the emission and absorption lines of sodium has a fractional width of $3{\cdot}8 \times 10^{-8}$ (natural line-width $2{\cdot}1 \times 10^{-8}$ and residual Doppler-width $3{\cdot}1 \times 10^{-8}$), and each hyperfine structure component of the absorption line is split up in the weak magnetic field into a multiplet with an effective magnetogyric ratio of 0·55 Bohr magneton for the six components.

In a second experiment the feasibility of Doppler scanning was studied by slowly rotating the absorber atomic beam A about an axis X (Fig. 2) and recording the transmission as a function of inclination θ of one beam with respect to the other.

By rotating the absorber atomic beam through an angle θ the absorption line is displaced by an amount $\Delta\lambda = \lambda\bar{v}/c \sin\theta = \lambda\bar{v}/c\ \theta$ for small θ, where $\bar{v}$ is the mean velocity of the atoms in the beam. Consequently, a measurement of the transmission in the range $\pm\theta$ corresponds to a measurement of the overlap of the emission and absorption lines as the wave-length difference varies from $-\Delta\lambda$ to $+\Delta\lambda$.

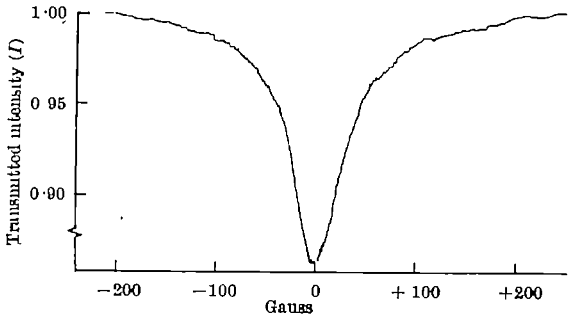

Fig. 3. Zeeman scan showing variation of transmitted intensity I with magnetic field applied to absorber A

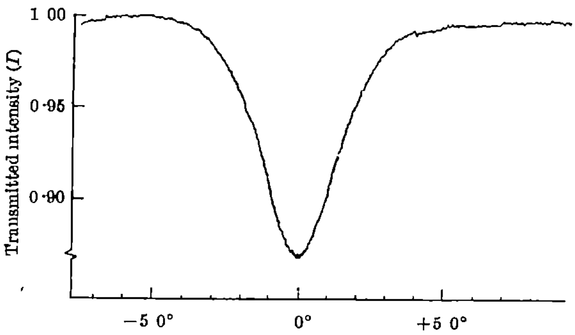

Fig. 4. Doppler scan showing variation of transmitted intensity I with inclination θ of absorber A with respect to emitter E; angular rotation through 1° corresponds to a wave-length change of 3×10^{-4} Å.

Unfortunately such a rotation produces a broadening and asymmetry[8] of the spectral line in addition to the Doppler displacement.

Fig. 4 shows such a transmission profile with a full width of $\theta = 3{\cdot}0°$. Assuming a mean atomic beam velocity[9] of 870 m.sec.$^{-1}$, this corresponds to a fractional width of $\Delta\lambda/\lambda = 15 \times 10^{-8}$.

This value exceeds slightly a theoretical estimation, taking into account the natural line-width, the residual Doppler broadening, the inclination broadening and the broadening resulting from the overlap of the hyperfine structure components of the $D1$ and $D2$ lines of sodium.

From these results it was deduced that the components of the sodium D lines had widths of approximately $\Delta\lambda/\lambda = 4 \times 10^{-8}$ corresponding to a resolution of the order of 10^{8}, but because of the overlap of the hyperfine structure components the resolution was reduced by a factor of 2.

We can conclude that the above method can, in rather special circumstances, produce very high resolutions and can be used to study spectral line displacements, spectral line profiles and integrated areas under a spectral line. The resolution is highest and the interpretation of the results is simplest if the atoms in the beam have a simple spectral line, that is, have no nuclear spin and are identical.

Among the obvious applications is the study of isotope shifts. A particularly attractive case would be the study of isotope shifts of mercury using one beam of a separated isotope of zero nuclear spin, for example, mercury-198.

An atomic beam of sodium is being used as a high-resolution spectroheliograph for the study of the profiles of the Fraunhofer absorption lines.

I wish to thank Mr. R. A. Dewar and Dr. R. L. Martin for their interest and the Directors of Imperial Chemical Industries of Australia and New Zealand, Ltd., for permission to publish this material.

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INFLUENCE OF MOLECULAR STRUCTURE ON THE LIQUID/LIQUID EXTRACTION OF THE CHLORO-COMPLEXES OF GALLIUM AND INDIUM WITH ALIPHATIC ETHERS

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THE determination of elements of low abundance in geological materials is of considerable interest in geochemistry, and very sensitive methods have been developed in recent years for the estimation of these trace elements Such techniques include neutron activation[1] and recent work on the combination of ion-exchange enrichment and spectrochemical analysis[2–5]. There is clearly a place for solvent extraction methods in the general scheme of analysis of elements of very low abundance, and this particular field seems to have been neglected in recent years.

An investigation has therefore been made into the extraction of gallium and indium (both elements of geochemical interest) from solutions of different concentrations of hydrochloric acid into a series of seven aliphatic ethers in an attempt to study more fully the factors involved in the extraction of these elements. (For reviews of general extraction procedures, see Irving[6], Martin and Holt[7] and Kitahara[8]).

Both indium and gallium are able to form ion-association complexes in the presence of hydrochloric acid, and in its very simplest form the mechanism is probably :

$$In^{3+} + 4\,Cl^- \rightleftharpoons InCl_4^- \qquad (1)$$

$$InCl_4^- + H^+ \rightleftharpoons H^+, InCl_4^- \qquad (2)$$

According to Bjerrum[9], the stability constant of the ion-pair formation (K) is dependent on the absolute temperature (t), the distance apart of the charge centres (d) and the dielectric constant of the solvent (e). In a simplified form the Bjerrum equation may be represented as follows :

$$K = \frac{C}{d.e.t.} \qquad (3)$$

where C is another constant.

At constant temperature it is obvious that K will depend on d and e, and part of the purpose of the present investigation was to attempt to establish the relative importance of these two factors.

If ion-association complexes only of the type shown in equation (2) were formed, then presumably the distance between the charge centres would always be constant and the extent of extraction into the ethereal phase would, at constant temperature, depend only on the dielectric constant of the solvent. However, oxonium solvents can play a special part in ion-association systems, since the basic character of the oxygen atom enables the solvent molecule to be incorporated in the co-ordination sphere of the metal[10]. We may therefore expect to have association complexes formed in the following manner :

$$In^{3+} + 6\,R_2O \rightleftharpoons In(R_2O)_6^{3+} \qquad (4)$$

$$In(R_2O)_6^{3+} + 4\,Cl^- \rightleftharpoons In(R_2O)_2Cl_4^- + 4\,R_2O \qquad (5)$$

$$In(R_2O)_2Cl_4^- + H^+ \rightleftharpoons H^+, In(R_2O)_2Cl_4^- \qquad (6)$$

In this case it would appear that steric effects should play an appreciable part in determining the value of the stability constant of the complex, since they will to a large extent determine the value of the charge distance d. A study of the extractability of gallium and indium into a series of homologous ethers should clearly furnish valuable data about which mechanism is operative in the extraction of these metals, provided the dielectric constants of the solvents are also known.

Experimental Procedure

A stock solution was prepared containing 0·02 mgm. of indium and the same quantity of gallium per ml. 1-ml. aliquots of this solution were transferred to a set of 50-ml. test-tubes, and varying amounts of hydrochloric acid and water were added to each so that the final volume was 5 ml. and the final concentrations of hydrochloric acid were : N, 2 N, 3 N, 4 N, 6 N and 8 N, respectively. Five ml. of an aliphatic ether (pre-equilibrated with the appropriate strength of hydrochloric acid) were then added to each test-tube and the contents shaken for 3 min. The phases were then separated and transferred to 50-ml. porcelain basins. Twenty mgm. of sodium chloride were then added as a matrix and the samples were taken to dryness. The residues were then loaded into graphite electrodes and the indium and gallium determined spectrochemically, using the magnesium impurity in the sodium chloride as internal standard. The spectra (2750–4680 Å.) were recorded on Kodak 103-0 blue-sensitive plates. The spectral intensities

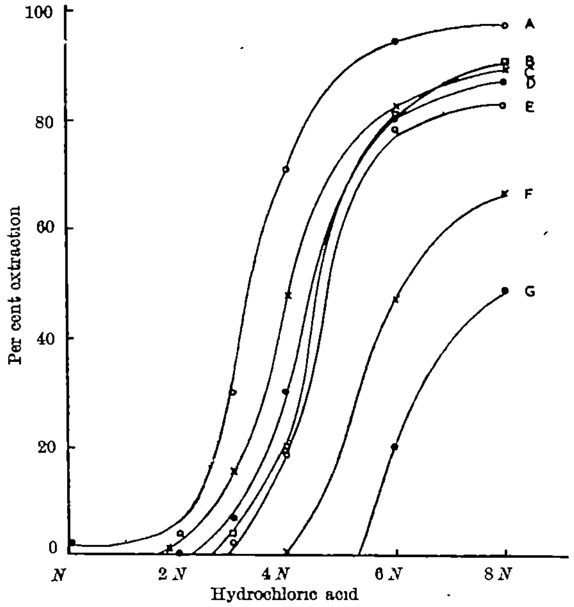

Fig. 1. Percentage extraction of gallium into aliphatic ethers from a range of concentrations of hydrochloric acid. A, ethyl ether ; B, methyl-hexyl ether ; C, ethyl-hexyl ether ; D, methyl-butyl ether ; E, *iso*propyl ether ; F, butyl ether ; G, amyl ether

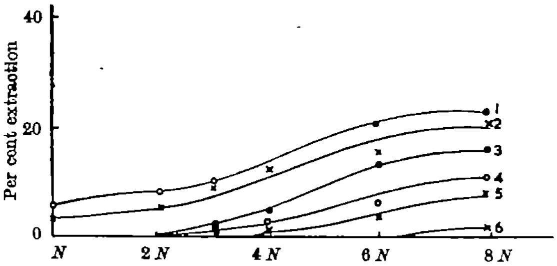

Fig. 2. Percentage extraction of indium into aliphatic ethers from a range of concentrations of hydrochloric acid. 1, ethyl ether; 2, methyl-hexyl ether; 3, ethyl-hexyl ether; 4, methyl-butyl ether; 5, *iso*propyl ether; 6, butyl ether

were measured and the percentage extraction hence determined.

The experiments were carried out on the following solvents: ethyl ether, *iso*propyl ether, butyl ether, amyl ether, methyl-butyl ether, ethyl-hexyl ether and methyl-hexyl ether. In every case the solvent was purified by distillation over sodium after removal of peroxides.

In Fig. 1 the percentage extraction of gallium is shown as a function of concentrations of hydrochloric acid for each of the seven ethers. The corresponding results for indium are shown in Fig. 2.

The dielectric constant for each ether was measured by the conventional condenser-resonance method[11], in which the sample is placed in a special cell between two silver electrodes and forms the dielectric of a condenser. A variable condenser is tuned to resonance with this system and the dielectric constants hence determined. These results are shown in Table 1.

Table 1. DIELECTRIC CONSTANTS OF ALIPHATIC ETHERS AT 20° C

Solvent	Dielectric constant
Ethyl ether	4·33
Methyl-butyl ether	4·20
Ethyl-hexyl ether	4·05
*Iso*propyl ether	4·04
Methyl-hexyl ether	3·90
Butyl ether	3·08
Amyl ether	2·82

An examination of Figs. 1 and 2 shows that, far from increasing with decreasing dielectric constants, the extractability of the chloro-complexes of indium and gallium actually decreases, and indeed at 4 *N* hydrochloric acid a plot of percentage extraction of gallium as a function of dielectric constant shows an approximately linear relationship of positive slope. Since the influence of dielectric constant appears to be slight, then, on the basis of Bjerrum's theory, the effect of the solvent co-ordination mechanism on the extraction of the complexes must be important, because this is the greatest factor which determines the distance apart of the charge centres. This co-ordination involves the question of steric hindrance where the size of the solvent molecule will obviously affect the factor d in the Bjerrum equation.

A study of the curves for gallium reveals a rather surprising fact (which is also obvious to lesser degree in Fig. 2), and this is the apparently greater efficiency of the asymmetric ethers for extracting the complexes compared with the symmetric compounds. In terms of extracting power, the three asymmetric ethers appear to be intermediate between ethyl and *iso*propyl ether, whereas steric considerations would tend to indicate an extraction potential certainly inferior to that of butyl ether. The symmetric ethers show a regular decrease in extracting power with increasing molecular weight, and the pattern is identical for indium and gallium.

This anomalous behaviour of the asymmetric ethers poses an interesting problem. A possible explanation may be that the greater dipole moments of these ethers play an important part in determining the extent to which they are able to enter the co-ordination sphere of the metal. It is considered that these higher dipole moments may lead to an increased electron density at the donor oxygen atom due to electrostatic forces within the molecule and hence increase the tendency of the solvent to co-ordinate with the appropriate metal.

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FATTY ACID PATTERN IN THE AORTA LIPIDS OF COCKERELS IN THE INITIAL STAGE OF CHOLESTEROL- OR STILBŒSTROL-INDUCED ATHEROSCLEROSIS

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IN the chicken, either the addition of cholesterol to the diet or the implantation of stilbœstrol pellets leads to a striking increase in blood lipids followed by the development of diffuse lipid infiltration of the inner parts of the aortic wall on which atheromata formation[1] is later superimposed. The mechanism of the atheromatous changes is poorly understood, although it is generally believed to be closely associated with cholesterol metabolism, which in turn is related to fatty acid metabolism[2]. In order to elucidate the relationship between fatty acid metabolism and the development of atheromatous changes a study was undertaken of the fatty acid composition of the cholesterol esters, triglycerides and phospho-

Table 1. THE FATTY ACID COMPOSITION OF THE LIPIDS OF AN INTIMA-MEDIA PREPARATION OF THE COCKEREL AORTA

Fatty acid	Cholesterol fatty acid Animal groups				Triglyceride fatty acid Animal groups				Phospholipid fatty acid Animal groups			
	A Control	*B* Cholesterol plus peanut oil	*C* Cholesterol, no oil	*D* Stilbœstrol, no oil	*A* Control	*B* Cholesterol plus peanut oil	*C* Cholesterol, no oil	*D* Stilbœstrol, no oil	*A* Control	*B* Cholesterol plus peanut oil	*C* Cholesterol, no oil	*D* Stilbœstrol, no oil
Saturated												
Pentadecanoic 15 : 0	1 8				0·8							
Palmitic 16 : 0	23 6	7 7	23 0	17·3	24·8	21 3	20·1	26·7	21·9	20·8	25 3	22·5
Heptadecanoic 17 · 0	3 1											
Stearic 18 . 0	13·4	2 1	5 2	8 1	19·9	15·2	16·1	10·1	21·3	15 6	19 6	20·4
Mono-unsaturated												
Palmitoleic 16 1	6 2	8·1	8 2	3 4	4·5	6·9	10 2	3·1	1·7	2·9	3·1	
Oleic 18 . 1	23 1	64·2	41·8	48 2	29·8	45·8	36·0	48·6	26·9	28·3	33·7	17 7
Di-unsaturated												
Linoleic 18 . 2	15·2	13·9	19 8	16 1	10 4	10·7	8 6	8·9	8·5	14·7	10 6	4·1
Poly-unsaturated												
Linolenic 18 3	1 8	1·3	0·7	2 5	4 1			1·4	0·2	1·1		
Arachidonic 20 . 4	1·6	1 1		4 4	1·7			1·2	11 8	11·5	7·7	
20–22 ?	6·4	1 5	1·2		3 5				6 5	5·1		16·1
24 ?												19·2

Group *A*, nine normal cockerels; Group *B*, three cockerels fed 1 per cent cholesterol plus 3 per cent peanut oil in the diet; Group *C*, two cockerels fed 1 per cent cholesterol without any oil; Group *D*, two cockerels implanted stilbœstrol pellets subcutaneously (no oil).

The tissues from the animals within the groups were pooled and the total fat extracted. The total lipids were separated into cholesterol esters, triglycerides and phospholipids on silicic acid.

Values are expressed as per cent of total fatty acid methyl ester

lipids of the blood and aorta lipids in cockerels with experimental atherosclerosis.

The present report deals with part of the analyses carried out on the aortic lipids. The findings may be significant regarding the pathogenesis of atherosclerosis, because they indicate changes in the oleic acid metabolism.

The cockerels used were a cross-breed of White Leghorn and New Hampshire, 5 months old at death.

Three groups of three normal cockerels were fed ordinary chick starter mash mixed with grain (1 : 1). The results from these three groups were averaged and are presented as group *A* in Table 1. Three animals (group *B*) were given a supplement to the mash consisting of 1 per cent by weight of cholesterol and 3 per cent peanut oil during 10 weeks before killing. Two animals (group *C*) were given 1 per cent cholesterol *without* any extra oil. Two animals (group *D*) were treated with stilbœstrol. Pellets of diethylstilbœstrol, each containing 10 mgm., were implanted subcutaneously. Eight implantations were performed at intervals of 10 days during a 10-week period. The last implantation was performed 5 days before death.

The aorta (including the brachiocephalic trunks) was dissected free from any visible fat and loose connective tissue. Approximately the inner half of the aortic wall (about 200 mgm. wet weight) consisting of intima and part of the media was isolated for fatty acid analysis.

In two of the aortas from the cholesterol-fed animals (group *B*) non-elevated, white-yellow areas were found in the intima. In one of the aortas from the stilbœstrol-treated animals (group *D*) atheromatous changes were also found.

Cockerels fed cholesterol and 3 per cent peanut oil (group *B*) showed a 270 per cent increase in cholesterol and a 14 per cent increase in phospholipid in the inner one-half of the aortic wall. Cockerels fed cholesterol without oil (group *C*) showed a 50 per cent increase in aorta cholesterol. Animals treated with stilbœstrol (group *D*) showed a 190 per cent increase in cholesterol and a 24 per cent increase in phospholipids of the aorta as compared with normal controls.

The aorta preparations within the three animal groups were pooled, cut into small pieces, and then extracted with ethanol/ether (3 : 1). The combined extracts were evaporated under nitrogen, dried in vacuum and dissolved in light petroleum.

The total lipids were separated on silicic acid into three fractions: cholesterol esters, non-esterified fatty acids plus triglycerides, and phospholipids[3].

The fatty acids of the three fractions were isolated and the methyl esters prepared by treating with diazomethane in ether. The fatty acid composition was determined by gas-liquid chromatography according to James and Martin[4] using a Pye argon chromatograph with an ionization chamber as detector. Because of the complexity of the fatty acid pattern, it was necessary to chromatograph the mixed methyl esters on two different stationary phases, a polar polyester (*LHC-R*-296)[5] and silicone oil[4]. In addition chromatograms were performed on the mixed esters after complete hydrogenation in order to obtain an accurate chain-length analysis. The analytical procedures used in addition have been described earlier[6]. The results of the analysis are summarized in Table 1. The most striking differences between animal groups *A*, *B*, *C* and *D* were found in the cholesterol ester fatty acid composition. The cholesterol esters of the control group *A* contained only 23·1 per cent oleic acid compared with 64·2, 41·8 and 48 2 per cent in animal groups *B*, *C* and *D* respectively. The stearic acid concentration on the other hand decreased from 13·4 per cent in the control group *A* to 2·1, 5·2 and 8·1 per cent in groups *B*, *C* and *D*. The linoleic acid concentration of the cholesterol esters showed no significant changes in the different animal groups.

In the triglycerides the percentage of oleic acid showed similar changes as in the cholesterol esters but less pronounced. The percentage of stearic acid showed, however, only a tendency to decrease and the percentage of linoleic acid showed no differences.

The fatty acid composition of the phospholipids in the groups was remarkably constant with the exception of group *D* where an increase in fatty acids belonging to group C_{20-24}? was found. The exact nature of these fatty acids has still to be determined.

In connexion with the pronounced changes in the oleic acid and stearic acid percentage in the cholesterol and triglyceride fatty acids found here two papers are relevant. Klein and Martin[7] have found an increase in the liver cholesterol oleate pool of cholesterol-fed rats. James *et al.*[8] have described an increase in oleic acid and a decrease in stearic acid percentage in the fatty acids of the acetone-soluble lipids of plasma from patients with coronary artery disease.

Concerning the influence of diet in these experiments, it is apparent that without any additions to the diet the stilbœstrol-treated animals showed changes in the cholesterol ester and triglyceride fatty acids similar to the changes found in the two cholesterol-fed groups. All three treated groups showed hyperlipæmia and cholesterol accumulation in the aorta.

The implications of these observations can be more properly assessed when the fatty acid composition of the plasma lipids is also known.

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GROWTH AND MORPHOGENESIS OF *STRIGA* SEEDLINGS

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Effect of Light, Gibberellic Acid and Kinetin on Growth and Morphogenesis of *Striga* Seedlings

BOTH facultative and obligate parasites occur in the family Scrophulariaceae. An example of the former is *Rhinanthus minor* Linn.[1], which can grow without the host, producing roots and shoots as a normal plant and even setting seed. The growth of such plants, however, is generally slower, and the plants may be stunted compared with those which become attached to a suitable host. Among the obligate parasites belonging to this group, species of *Striga* are well known for attacking grain crops in tropical and sub-tropical countries. The seeds of these parasites require a germination stimulus which is produced by the roots of host plants. Saunders[2] demonstrated that leachings from sand in which the roots of maize had been growing could be used to germinate *S. asiatica* (= *S. lutea*) and to grow the seedlings for a short period without the host. In the present experiments a solution produced by growing a susceptible variety of *Sorghum vulgare* in small pots of sand for a number of days and eluting with water was used to germinate seeds of *S. asiatica*. In this solution seedlings produced a slender unbranched radicle (Figs. 1 and 2) up to 4 mm. long, but generally less, before the food reserves in the seeds were used up and the seedlings died. The radicle was usually strongly curved at first or grew in the form of a spiral, and root hairs were not generally produced although a few short hairs sometimes grew towards the radicle tip of older seedlings, and occasionally an abnormal, highly deformed seedling which sprouted root hairs was produced in such cultures.

The effects of light and the growth substance gibberellic acid and kinetin on this morphogenic pattern have been studied. In the growth-substance experiments young germinated seedlings (between 15 and 30 in each replicate) were transferred to small culture vessels in 1 ml. of the germination solution. To this was added 4 ml. of either water or the particular hormone solution under investigation. The cultures were maintained in the dark except for short periods when they were examined. Growth was recorded by means of camera-lucida drawings or photographically. In the light experiments seedlings were exposed to light of different qualities or kept in the dark. A No. 2 Photoflood lamp was used as a light source. The light was filtered through an infra-red filter and a water filter circulated with air-cooled water. This arrangement permitted the treatment of seedlings with fairly high-energy light without heating; light of restricted wave-length was obtained by the use of coloured glass filters. These were matched for equal energy transmission (using a simple thermopile and galvanometer) with a series of neutral filters made by reducing fogged (black) photographic film to the desired density. The accuracy of the thermopile and galvanometer permitted only approximate matching of the filters. The unfiltered light (about 2,500 ft.-candles at the level of the culture vessel) gave a galvanometer reading of 8 units, while the densest filter used gave a reading of 1·5 units. The neutral filters were used to adjust the other filters to give this reading on the thermopile galvanometer. Using this arrangement, only one light treatment could be studied at a time so that each result is compared with a separate dark control. The results of the light experiments are shown in Table 1.

The major effects of light were: (1) reduced extension growth of the radicle; (2) increased diameter; (3) the production of root hairs; (4) the occasional proliferation of cells into a branch-like swelling of the radicle. The number of root hairs shown in Table 1 refers only to those that were countable in a profile view of the seedlings, the actual numbers being considerably higher than this (some

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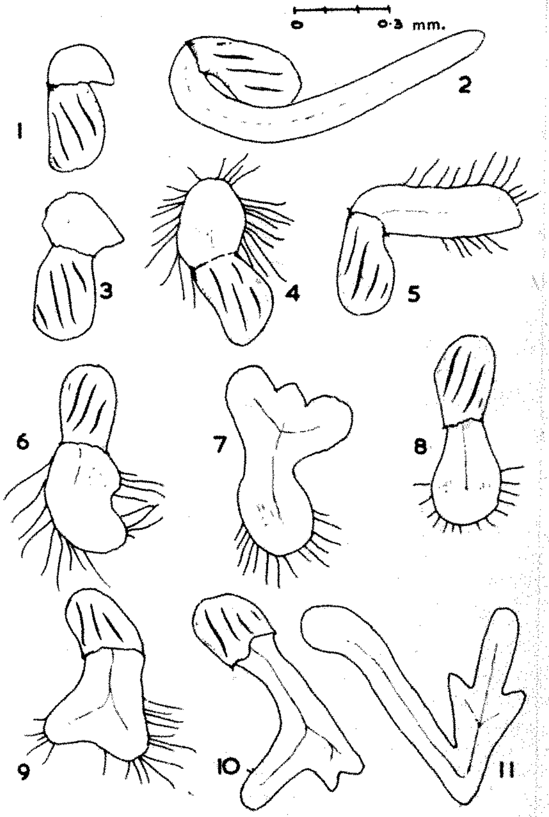
Table 1. EFFECT OF LIGHT OF DIFFERENT QUALITIES ON DEVELOPMENT OF THE *Striga* RADICLE

Light quality	Transmission range of filter (mμ)	Max. transmission (mμ)	Extension growth light growth/dark growth	Diam. light/dark	Root hairs (av. per seedling over controls)
White	—	—	0·31	1·8	8·1
Blue	*c.* 350–650	490	0·45	1·6	7·4
Green	470–650	540	0·81	1·3	2·1
Red	600–*c.* 1,100	*c.* 650	0·68	1·3	2·5
Far-red	700–*c.* 1,100	*c.* 770	1·06	1·0	0

typical effects of light are illustrated in Figs. 3–5). The most effective wave-lengths in producing these morphogenic effects were in the blue region of the spectrum and to a lesser extent in the red. Far-red irradiation produced a slight, though not significant, increase in extension growth and was without formative effects. It was not tested whether far-red could reverse the effect of fixed exposures to light of the other wave-lengths. While the effects of red and far-red in many plant growth processes are reversible through the action of red and far-red absorbing forms of a special pigment system (for example, seed germination, flowering, internode extension, hypocotyl curvature, anthocyanin synthesis)[3–6], some plants are reported to be more sensitive to blue irradiation[7] in flowering and growth responses. According to Hendricks and Borthwick[6], photomorphogenic responses to blue light also arise from the absorptions of the photomorphogenic pigment. That the situation is probably more complex is indicated by the results of Stozwijk and Zeevaart[8] in which both blue and far-red were found to be more effective than red in promoting flowering of the long-day plant *Hyoscyamus niger*, while generally flowering of long-day plants appears to respond to red (and to be inhibited by far-red).

Gibberellin and kinetin are known to simulate certain effects of light ; both promote leaf expansion and seed germination, while gibberellin simulates the effect of red light on the flowering of long-day and short-day plants (see review by Brian[9]). Brian postulates the action of red light in raising the level of a gibberellin-like hormone which may in some way affect the auxin status of plants. The effects of these substances on the development of *Striga* seedlings are shown in Table 2.

Kinetin had a striking effect on root hair production and radicle growth resembling the effects of light (Figs. 6–9). Root hair production and length of hairs were greatest at 1 p.p.m. and least at 100 p.p.m. At the 1 p.p.m. level kinetin induced branching in the radicle of some seedlings, with pro-vascular strands passing from each branch (Fig. 9). At the 10 p.p.m. level, and to some extent at the 100 p.p.m. level, shoot development shown by expansion of the cotyledons and enlargement of the plumule were stimulated. (The effects of kinetin on root hair production and shoot development have been previously reported by Worsham *et al.*[10], who also report that kinetin at certain levels promotes germination of the parasite seeds. The resemblance of the effects of kinetin and light, however, has not to my knowledge been previously reported.)



Figs. 1 and 2, stages in the growth of *Striga asiatica* seedlings in the dark without growth substance additions ; Figs. 3–5, some effects of light on *Striga* seedlings ; Figs. 6–9, effects of kinetin at various levels ; Figs. 10 and 11, some effects of gibberellic acid on seedling development. (Camera-lucida drawings)

Table 2. EFFECT OF GIBBERELLIC ACID (GA) AND KINETIN ON DEVELOPMENT OF *Striga* SEEDLINGS

Treatment	Extension growth of radicle (mm.)	Diam. of radicle (mm.)	Root hairs (av. per seedling)	Branching of radicle (per cent seedlings)	Shoot expansion (per cent seedlings)
Control	1·08	0·10	1·4	0	0
GA					
0·05 p.p.m.	0·98	0·11	0·8	0	0
0·2 p.p.m.	0·63	0·14	2·3	0	0
1 p.p.m.	0·57	0·15	2·0	35	30
5 p.p.m.	0·50	0·13	0·8	50	35
25 p.p.m.	0·51	0·12	0·6	10	45
Kinetin					
1 p.p.m.	0·32	0·19	30	25	0
10 p.p.m.	0·29	0·22	18	0	65
100 p.p.m.	0·31	0·22	17	0	15

The action of gibberellic acid resembled that of kinetin in inducing shoot development and branching of the radicle in some seedlings. With this substance, however, the radicle branches were more elongated and extension growth was not reduced to the same extent (Figs. 10 and 11) : gibberellic acid had no significant effect on root hair production under these conditions, although it has been reported to promote root hair formation in excised pea roots[11]. Shoot development was pronounced with gibberellic acid at 5 and 25 p.p.m. and differed from kinetin in showing definite internode extension (Fig. 11). Clearly gibberellic acid does not simulate the effect of light in the development of *Striga* seedlings as does kinetin.

The morphogenic changes induced by light and these growth substances are of particular interest, since development in the dark beyond the stage of radicle extension in *Striga asiatica* normally only occurs if the parasite becomes associated with a suitable host plant. The production of shoots and the stimulation of root branching by kinetin and gibberellin indicate that the parasite probably depends on a supply of growth factors of this type from the host for development, unlike the facultative parasites belonging to the same family (for example, *Rhinanthus*). Shoot development has never been observed to occur with light treatment alone. Various attempts have been made to prolong the growth of *S. asiatica* in culture by providing mineral nutrients and certain vitamins and organic compounds (for example, thiamin, nicotinic acid, pyridoxine, adenine), as well as kinetin, gibberellic acid and light; but chlorophyll development and growth beyond the stages indicated have never occurred.

Influence of the Host Rhizosphere on the Morphogenesis of *Striga* Seedlings

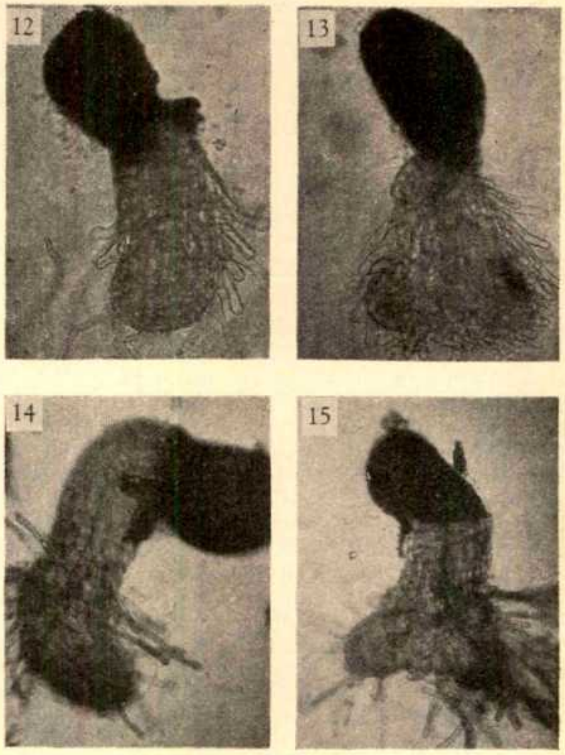
USING an agar culture technique, studies have been made on the development of the radicle of the root parasite *Striga asiatica* (= *S. lutea*) in the rhizosphere of a susceptible host plant. The method used was as follows: parasite seeds were treated with moisture on damp filter paper for about 10 days before use. The seeds do not normally germinate except in the presence of an exudate from the roots of a host plant (for example, sorghum, maize), and a period of moisture treatment is necessary for optimum germination. After moisture treatment the parasite seeds were mixed into an autoclaved 1 per cent agar solution at about 40–45° C., pipetted into 6-cm. Petri dishes and allowed to set. 10 c.c. of the agar seed suspension was pipetted into each dish (that is, several hundred seeds). Immediately after pipetting the agar, one host seedling (*Sorghum vulgare* var. Light Mori, from Nigeria) was rinsed briefly with sterile water and planted in each dish. The host seeds were surface sterilized with bromine water prior to germination, and the radicles at the time of planting were about 2 cm. long. The dishes were incubated in the dark at room temperature at a slight incline to induce the host radicles to grow across the dishes. Five or ten replicates were used, and the experiment was repeated on a number of occasions.

A germination factor, diffusing from the host radicle, caused the parasite seeds to germinate. Germination varied considerably between the replicates, but always increased with proximity to the host radicle. In addition to germination, growth-rate and morphology of the radicle varied strikingly with distance from the host radicle. The development of the parasite radicle in 4-mm. zones about the host root was measured by means of camera-lucida drawings. In the nearest zone the rate of extension growth of the radicle was very low, the radicle undergoing considerable lateral expansion and producing root hairs (Fig. 12). Occasionally branching of the radicle occurred (Fig. 13). In this zone the host root showed a definite positive chemotropic influence over the growth of the parasite radicle. In the second zone (4–8 mm. from the host root) the rate of extension growth of the parasite radicle was considerably increased, and lateral expansion and the production of root hairs were reduced. Similarly, in the third zone (8–12 mm. from the host root) the rate of extension growth was further increased and lateral expansion and root hair formation reduced. These results are listed in Table 3.

Table 3. MORPHOGENIC EFFECT OF THE HOST RHIZOSPHERE ON THE RADICLE OF THE ROOT PARASITE *Striga asiatica*

Distance from host root	Root hairs* (average per seedling)	Extension growth (mm.)	Diameter (mm.)
0–4 mm.	12·2	0·45	0·16
4–8 mm.	2·7	0·95	0·13
8–12 mm.	1·5	1·25	0·10

* Only the root hairs visible in profile were counted; the actual numbers were considerably higher.



Figs. 12 and 13, typical morphogenic effects of the host rhizosphere; Figs. 14 and 15, effects of kinetin at a concentration of 1 p.p.m.

The results show that a factor having a strong morphogenic influence on the development of *Striga* seedlings is present in the host rhizosphere and is probably secreted by the host-plant root. It is of interest that similar effects on the development of *Striga* seedlings can be induced in cultures without the host by treatment with light (particularly blue light and to a lesser extent red), and with the light-simulating hormone kinetin [6-(2-furfuryl) amino-purine] (ref. 10 and previous communication). Figs. 14 and 15 show typical effects of kinetin at a level of 1 p.p.m. on *Striga asiatica* seedlings. Root hair production and lateral expansion of the radicle are stimulated and extension growth is strongly reduced, while some seedlings are induced to branch in a way very similar to that which occurs in the region of the host root. At higher levels (10 and 100 p.p.m.) shoot development was stimulated by kinetin. No shoot development was ever observed to result from exudates in the rhizosphere of the host, however, although shoot development commences shortly after contact is made with the host root.

Worsham *et al.*[10] have shown that certain 6-substituted amino-purines, including kinetin, stimulate the germination of *Striga asiatica* seeds at levels ranging from 5 to 25 p.p.m., and can replace the natural germination stimulant excreted by the host roots. The natural germination stimulant has not, to my knowledge, been identified, although chromatography of concentrates prepared from water in which the roots of sorghum had been growing revealed a highly active zone close to the solvent front[12]. This stimulant was thought to be similar to the *Orobanche* seed stimulant from linseed roots, which apparently also stimulates the germination of *Striga* seeds and has a similar R_F value[13]. Degradative work on the *Orobanche* stimulant, however, has shown it to have a small nitrogen value (0·5 per cent)[14] and therefore it does not appear to be a purine compound. A more recent investigation[15] on the *Striga* and *Orobanche* germination stimulants from maize roots shows that root extracts, and aqueous solutions in which the roots have been suspended, contain a number of stimulating substances for these seeds, including a water-soluble component and a number of ether-soluble components. Chromatographic analysis indicates that the factors from maize roots causing optimal germination of *Striga* and *Orobanche* seeds are different. Traces of solutions from linseed and sorghum roots acted synergistically with maize root preparations, suggesting that linseed, sorghum and maize may all produce a similar complex of stimulants, differing only in the proportions of the different constituents.

In view of the present results and the demonstration by Worsham *et al.*[10] that 6-substituted aminopurines can stimulate the germination of *Striga* seeds, it would seem possible that one of the components of the germination complex may be a kinetin-like compound, which both stimulates the germination of the parasite seeds and induces morphogenic changes in the seedlings. In this case the concentration required for germination would be considerably less than that required for the appearance of morphogenic effects.

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ACTION POTENTIAL WITH AN ARTIFICIAL *p*H-MUSCLE

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An artificial *p*H-muscle[1] consisting of a three-dimensional network, made from about equal quantities of polyvinyl alcohol and polyacrylic acid, has previously been shown to dilate and to contract reversibly when sodium hydroxide and hydrochloric acid was added alternately to its embedding medium, for example, 1×10^{-3} *N* sodium chloride solution. It was consequently demonstrated for the first time with the artificial cross-striated *p*H-muscle[2] that the chemically induced dilations and contractions are paralleled by chemical equilibrium changes if the length of the filaments are changed mechanically. These chemical equilibrium changes were also shown to occur in an analogous way with the homogeneous *p*H-muscle[3], the redox-[4] and the ion-precipitation-muscle[5]; and they are the basis for a transformation of chemical into mechanical energy by means of these systems and the establishment of corresponding general and quantitative relationships: the 'teinochemical principle'[6].

In the course of comparing the behaviour of the artificial *p*H-muscle with the properties of the natural muscle, the potential between the inside of the artificial *p*H-muscle and its embedding medium was determined in a series of preliminary experiments, during: (1) an initial state of thermodynamic equilibrium, and then sequentially in (2) the various stages following a contraction or dilation stimulus. The latter potentials (2) will be seen to vary with time and may be referred to as action potentials, being characteristic for a system in which the dilations or contractions with the corresponding time-dependent interior changes are at the start, or in the subsequent course, of development.

High-polymer heat-vulcanized gel strips, consisting in the dry state of equal parts of polyvinyl alcohol and polyacrylic acid, were prepared and the polyacrylic acid content was partially (for example, 20 per cent) neutralized by placing the foils in an appropriate quantity of dilute aqueous sodium hydroxide. The partially neutralized gel strips were afterwards embedded in an aqueous 1×10^{-3} *N* sodium chloride solution, the volume of which was less than 100 times the swollen gel volume (for example, 4·0 cm. × 3·0 cm. × 1·0 cm.). After complete equilibrium with respect to both swelling and *p*H had been established between the swollen gel strip and its embedding medium, a small quartz capillary (filled with agar–agar and saturated potassium chloride, and which was connected to a saturated potassium chloride–calomel electrode) was inserted into the swollen gel strip; the capillary end of a second and similar electrode assembly was introduced into the embedding medium. The electrical circuit was completed by connecting the metallic ends of the saturated potassium chloride–calomel electrodes to a calibrated Polymetron type 42*B* millivolt meter. A more detailed description of the experimental apparatus, conditions and procedure will be given elsewhere[7].

In the ensuing time–potential curves (Figs. 1–3*a*, *b*) the potential of the electrode inside the gel relative to the electrode in the embedding medium is represented by the ordinate, the time by the abscissa. On inserting the internal electrode into the chemically stretched polyvinyl alcohol plus polyacrylic acid swollen gel strip (that is, a gel the polyacrylic acid content of which had been partially neutralized with aqueous sodium hydroxide), a potential is obtained which is represented by point *A* of curve *ABCD* in Fig. 1. This potential, which is constant in time (cf. part *A*–*B* of curve *ABCD* in Fig. 1) and negative with respect to the embedding medium, corresponds to a state of equilibrium between the gel strip and the embedding medium; it is identical with the Donnan potential[8] existing at the surface of the gel and the surrounding fluid.

With the addition of hydrochloric acid (for example, 3 c.c. of 0·1 *N*) to the embedding medium of the partially neutralized polyvinyl alcohol plus polyacrylic acid swollen gel strip (point *B* of curve *ABCD* in Fig. 1) a contraction stimulus is applied to the gel. The potential rises rapidly from a negative value (point *B* of curve *ABCD* in Fig. 1) to a highly positive value (point *C* of curve *ABCD* in Fig. 1) and then gradually decreases with increasing time to a final value (point *D* of curve *ABCD* in Fig. 1). The potential drops to zero (example, point *D* in Fig. 1) if the amount of hydrochloric acid added to the embedding medium is sufficient to convert all of the sodium polyacrylate in the gel to polyacrylic acid.

The potentials represented by points *A*, *B* and *D* in Fig. 1 are equilibrium potentials; they are completely accounted for by the respective ion concentrations in the gel and the embedding medium. The potentials between points *B* and *D*, in particular the potential at point *C* of Fig. 1, correspond to a non-equilibrium state of the system. Point *C* of curve *ABCD* in Fig. 1 (the spike of the overshoot of the time–potential curve) represents the potential of the chemically stimulated partially neutralized polyvinyl alcohol plus polyacrylic acid swollen *p*H-muscle at the point of its first major perceptible contraction (ref. 7*a*). Qualitatively, the positive potential value of the gel at point *C* (Fig. 1) is due to the hydrogen ions moving from the acidified embedding medium into the interior of the gel, the product of concentration and mobility of the hydrogen ions being the highest of all ions that are present at the interface of the gel and therefore dominating the potential. In the subsequent time period (between point *C* and point *D* of Fig. 1) the details of the explanation become more complicated; the concentration gradients due to ionic diffusion both inside and outside the gel, the reaction of the sodium polyacrylate with the hydrochloric acid, and the general or local contraction of the gel, will develop and may lead to local potential gradients of different sign and magnitude. The observation of a strong peak of positive potential after the administration of the chemical contraction stimulus is the main feature of curve *ABCD* of Fig. 1.

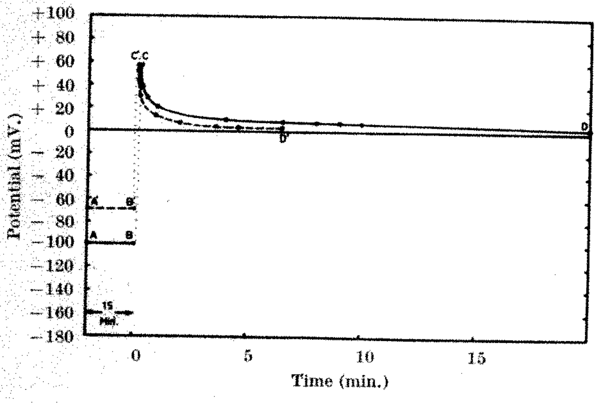


Fig. 1. Time–potential curves produced at 20° C. by hydrochloric acid on: (1) Partially neutralized swollen polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1 × 10^{-3} *N* sodium chloride solution (curve *ABCD*); addition of hydrochloric acid to the embedding medium at time zero, corresponding to point *B*; first major perceptible contraction begins at point *C* and accompanies the time–potential curve *C*–*D*
(2) Acidified swollen non-contractile polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1 × 10^{-3} *N* sodium chloride solution (broken curve *A'B'C'D'*); addition of hydrochloric acid to embedding medium at time zero, corresponding to point *B'*; no perceptible contraction of this (acidic) gel strip

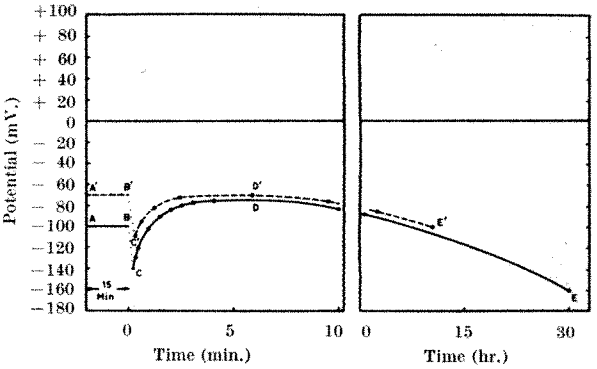


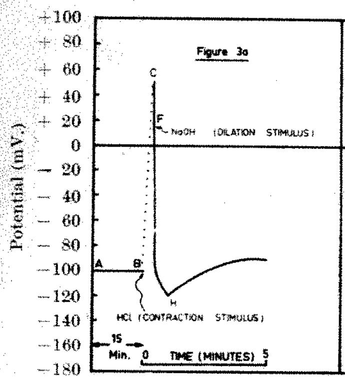
Fig. 2. Time–potential curves produced at 20° C. by sodium hydroxide solution on:
(1) Partially neutralized swollen polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1 × 10^{-3} *N* sodium chloride solution (curve *ABCDE*); addition of sodium hydroxide solution to the embedding medium at time zero, corresponding to point *B*; first major perceptible dilation of gel strip begins at point *C* and accompanies the time–potential curve *CDE*
(2) Acidic swollen non-contractile polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1 × 10^{-3} *N* sodium chloride solution (broken curve *A'B'C'D'E'*); addition of sodium hydroxide solution at point *B'*; first major perceptible dilation (relaxation) begins at point *C'* and ends at point *E'*
Serrated edges of time–potential curves represent time-lapse

The effect of hydrochloric acid on an acidic swollen non-contractile polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1 × 10^{-3} *N* sodium chloride solution is represented by curve *A'B'C'D'* and text in Fig. 1; a more detailed discussion will appear elsewhere[7].

When a partially neutralized or acidic polyvinyl alcohol plus polyacrylic acid gel strip, again prepared and embedded in 1 × 10^{-3} *N* sodium chloride solution as in the previous experiments, is given a dilation stimulus by the addition of sodium hydroxide solution (for example, 3 c.c. of 0·1 *N*) to the embedding medium, an immediate potential peak of the opposite sign is observed (that is, the internal gel electrode becomes more negative relative to the outer electrode in the embedding medium (Fig. 2)).

The peak of the negative potential (points *C'* and *C* of Fig. 2, that is, the point where the alkali-stimulated gel begins its first major perceptible dilation) is followed by a drop of the absolute value of the negative potential and after a long period by a moderate increase of the negative potential value towards a final equilibrium value which again can be explained by the state of gel swelling, and of the ion concentration both inside and outside the gel present in the final equilibrium state: curves *ABCDE* amd *A'B'C'D'E'* of Fig. 2.

It is interesting to note that a contraction stimulus (produced by the addition of acid to the embedding medium) followed a short time later by a dilation stimulus (a relaxation stimulus produced by the



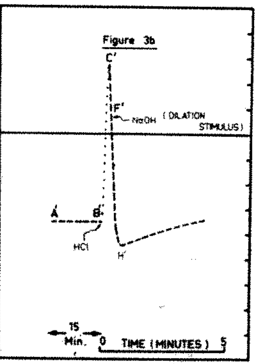


Fig. 3*a*. Time–potential curve for the effect of hydrochloric acid (contraction stimulus) followed a short time later by the addition of sodium hydroxide solution (dilation stimulus) on a partially neutralized polyvinyl alcohol plus polyacrylic acid swollen gel strip embedded in 1×10^{-3} N sodium chloride solution; this curve is a superimposition of curve *ABCD* of Fig. 1 on curve *ABCD* of Fig. 2 with a small time shift of the latter curve. Hydrochloric acid added at point *B*; first major perceptible contraction would be observed, under the present experimental conditions, at point *C*. Sodium hydroxide solution added at point *F*; dilation would begin shortly after point *F* and continue until the final equilibrium point. The significance and means of varying the slope and duration of the line *CFH*, and the recovery period beyond point *H*, will be presented elsewhere (ref. 7)

Fig. 3*b*. Time–potential curve for the effect of hydrochloric acid followed a short time later by the addition of sodium hydroxide solution (dilation stimulus) on an acidic swollen non-contractile polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1×10^{-3} N sodium chloride solution; this curve is a superposition of curve *A'B'C'D'* of Fig. 1 on curve *A'B'C'D'* of Fig. 2 with a small time shift of the latter curve. No perceptible contraction of gel strip on addition of hydrochloric acid. Remainder of legend as for Fig. 3*a*

addition of alkali to the embedding medium) of the swollen partially neutralized gel strip will produce a time–potential curve (for example, Fig. 3*a*) which has many of the characteristics of the time–potential curve (that is, of the action–potential curve) of various biological tissues[9]; in fact, the same curve of Fig. 3*a* is produced by a superimposition of curve *ABCD* in Fig. 1 on curve *ABCD* of Fig. 2 with a small time-shift of the latter curve.

Fig. 3*b* shows the effect of the addition of hydrochloric acid followed later by the addition of sodium hydroxide solution on a swollen non-contractile acidic polyvinyl alcohol plus polyacrylic acid gel strip embedded in 1×10^{-3} N sodium chloride solution; this will be discussed at greater length elsewhere[7].

A comparison of the resting, action and recovery potentials observed with various biological tissues[9] and the potentials observed with the high-polymer pH-muscle reveals some obvious formal analogies. Further ways of producing by appropriate means in other artificial high-polymer systems (for example, cross-striated pH-muscle, redox- and ion-precipitation-muscle) potentials dependent on time and similar to the different action–potential curves of various biological tissues will be given elsewhere[7]. Subsequent papers will also include the more complicated time–potential curves for high-polymer systems when acids and/or bases and other chemical substances are introduced either separately or in combination into the interior of the neutralized or acidic gel and/or the embedding medium at the same time or after a specific time lag, plus a discussion of the chemical and physical properties which influence the specific time–potential curve[7].

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PHOTOGRAPHIC CUMULATION OF RECURRENT RESPONSE POTENTIALS

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INTERMITTENT illumination of the human eye elicits a corresponding succession of response potential waves in the electroretinogram. Lord Adrian[1] has shown that these waves have negative and positive components the form of which is related to the frequency and intensity of the stimulating flashes. Much interest now centres on isolating the small responses characteristic of the photopic or colour-specific systems of the eye[2,3]. Unfortunately, a relatively large background of electrical 'noise' is usually present in an actively responsive system such as the eye. Often it is true that the signals generated in response to the light are smaller than the 'noise' that is unrelated to the stimulus.

One time-worn procedure for separating evoked potentials from irrelevant ones has been the comparison of several samples of record made under conditions such that they ought, apart from contamination, to appear identical. This is helpful; but the unaided eye is incapable of making these comparisons in a completely unbiased manner, and most workers agree that the required integration and abstraction would be better carried out using some more objective procedure. The use of electronic computers offers an exciting prospect of unheard-of sensitivity in the recording of biological potentials[4]. The apparatus, however, is complex and costly. It is usually not feasible to occupy such elaborate equipment throughout the routine, day-after-day exploration of individual electrophysiological phenomena.

The present article describes a simpler procedure, one which stores and cumulates its information on photographic film during successive exposures. We have applied the method specifically to the registration of the electroretinogram and have been able to identify components with amplitudes of less than one microvolt. The principle appears easily adaptable to any of a broad range of problems in which the response is stimulus-locked.

Our apparatus for cumulating electrophysiological responses is illustrated in Fig. 1. Light from a small tungsten filament is collected by a spherical mirror, directed through a rectangular aperture, and focused on the mirror of a bifilar oscillograph galvanometer having a natural frequency of 4,000 cycles/sec. A collimating lens in front of the galvanometer provides an image of the aperture in the form of a uniformly illuminated, rectangular patch of light. The rays forming this patch are reflected downward by a right-angle prism on to a horizontal diaphragm pierced by two rectangular windows. Any deflexion of the galvanometer mirror causes a proportional amount of light to traverse window *N* or window *P*, depending on the direction of movement of the mirror. In the null position the light falls between the two windows, illuminating neither.

When the eye is stimulated by a flash of light the negative and positive phases of the ensuing retinal potential, as amplified and fed into the galvanometer, are represented in the amounts of light traversing windows *N* and *P*. To record this, a cylindrical drum is wrapped with photographic film and rotated on a shaft directly below the windows of the diaphragm. A cylindrical lens focuses the light coming through the two windows on to the drum as two narrow lines of light. These, as the drum rotates, make parallel ribbons of exposure on the film, representing windows *N* and *P*.

To use the device for the cumulative recording of evoked potentials it is only necessary to synchronize the stimulus with the rotation of the drum. We have mounted a flicker-vane with four open sectors on the same shaft as the recording drum. Light passing through the open sectors provides the eye with a succession of flashes each of which evokes a response actuating the oscillograph galvanometer. A shutter is then opened, admitting the light to the rotating film for a fixed period of time—long enough to include the responses to several hundred flashes. The developed film appears as in Fig. 2. Variations in density indicate the existence of synchronous responses; randomly occurring 'noise' only adds a nearly uniform darkening of the two tracks. In our system, with four flashes per revolution, we see four cycles of density variation on each pair of tracks.

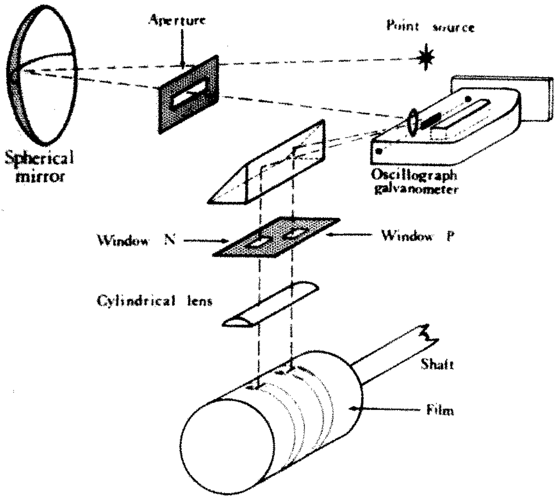

Fig. 1. The optics of the photographic cumulator—a schematic representation

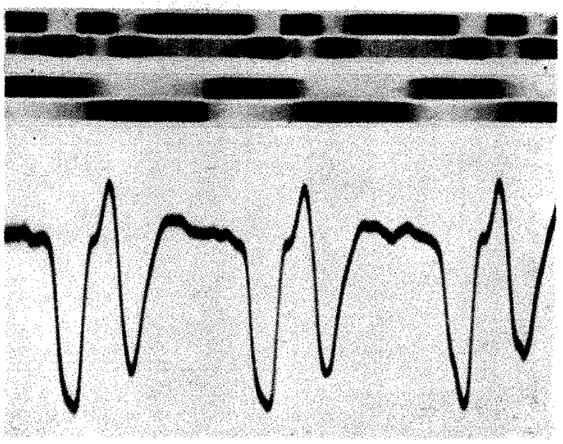

Fig. 2. Film records of cumulated electroretinal responses at two different flicker-rates, 30 per sec. (bottom pair) and 10 per sec. (top pair). The latter pair has been scanned with a recording densitometer to yield the 'average response' pattern shown beneath. Three response cycles are shown, each one being a cumulation of 75 individual responses

To 'read' our film records we use a double-beam densitometer and record the difference in density, between the two tracks, as a function of phase position. An oscillographic record of the output of the densitometer appears below the film in Fig. 2. This record shows the main features of the response without serious contamination by background 'noise'. Calibration of the system shows that it is reasonably linear despite the fact that film density is logarithmically related to exposure. This is because the method is one in which the final result is given by relatively small differences in densities that are nearly equal between the two tracks. For more exact determinations of the absolute potential-levels it would be necessary to develop the negative under conditions resulting in a photographic gamma of unity, printing a film positive under the same conditions, and finally scanning this positive with photocells having a linear response to light intensity.

The optical method of cumulation has proved its ability to reduce irrelevant potential 'noise' and hence to reveal stimulus-locked responses of very low amplitudes. We are now able to record electroretinographic responses to light that is flickering at a rate considerably higher than the critical frequency of fusion as seen by the observer. The method has the disadvantage that conditions of exposing, developing and scanning the photographic film must be given careful consideration and selected for appropriateness. It has the advantages of simplicity, reliability and low cost, coupled with the fact that there is continuous rather than discrete time-sampling of the response potentials.

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LETTERS TO THE EDITORS

PHYSICS

High Strength Glass

It was reported recently[1] that the major stress-raising defects on the surface of freshly drawn fibres of glass are visible, local defects which arise through the interaction of the hot glass with dust particles or other contamination. In that work very approximate values of the tensile stress which small areas of the surface, free from visible defects, would withstand without fracture were obtained. This communication is to report some results of further experiments to measure the actual breaking strengths of 'defect-free' samples of several commercial glasses.

Two methods have been used to prepare fibres of diameter 350–700μ which are free from visible defects, and these have been tested in 3-point bending using a gauge-length of 7 mm. and knife edges covered with polytetrafluoroethylene. For the first series of experiments lengths of fibre were produced by hand drawing from sections of ¼-in. diameter commercial rod which had been etched in 20 per cent hydrofluoric acid immediately before drawing. The centre of the rod was heated in an oxygen–coal-gas flame until quite fluid, removed and allowed to cool slightly before drawing. In a series of draws occasional defects are to be found, so that this simple procedure is not ideal. However, it has been found possible to eliminate from the results the more obvious cases of samples weakened by the presence of a defect, as described here. For the second series of experiments no precautions were taken to prevent the formation of defects during the heating of the rods before drawing. Instead, they were removed from the surface of the fibres after drawing by etching in hydrofluoric acid.

A sufficiently prolonged attack leaves smooth saucer-shaped pits in the fibre surface at the sites of the original defects. For samples of ×8 glass, etching for 5 min. in a 10 per cent solution of hydrofluoric acid was sufficient to achieve this, and reduced the diameter by approximately 60μ. Samples etched for 10 min. in the same solution did not show significantly different strengths. Similar treatment of 'Pyrex' specimens removed much less material; the strengths

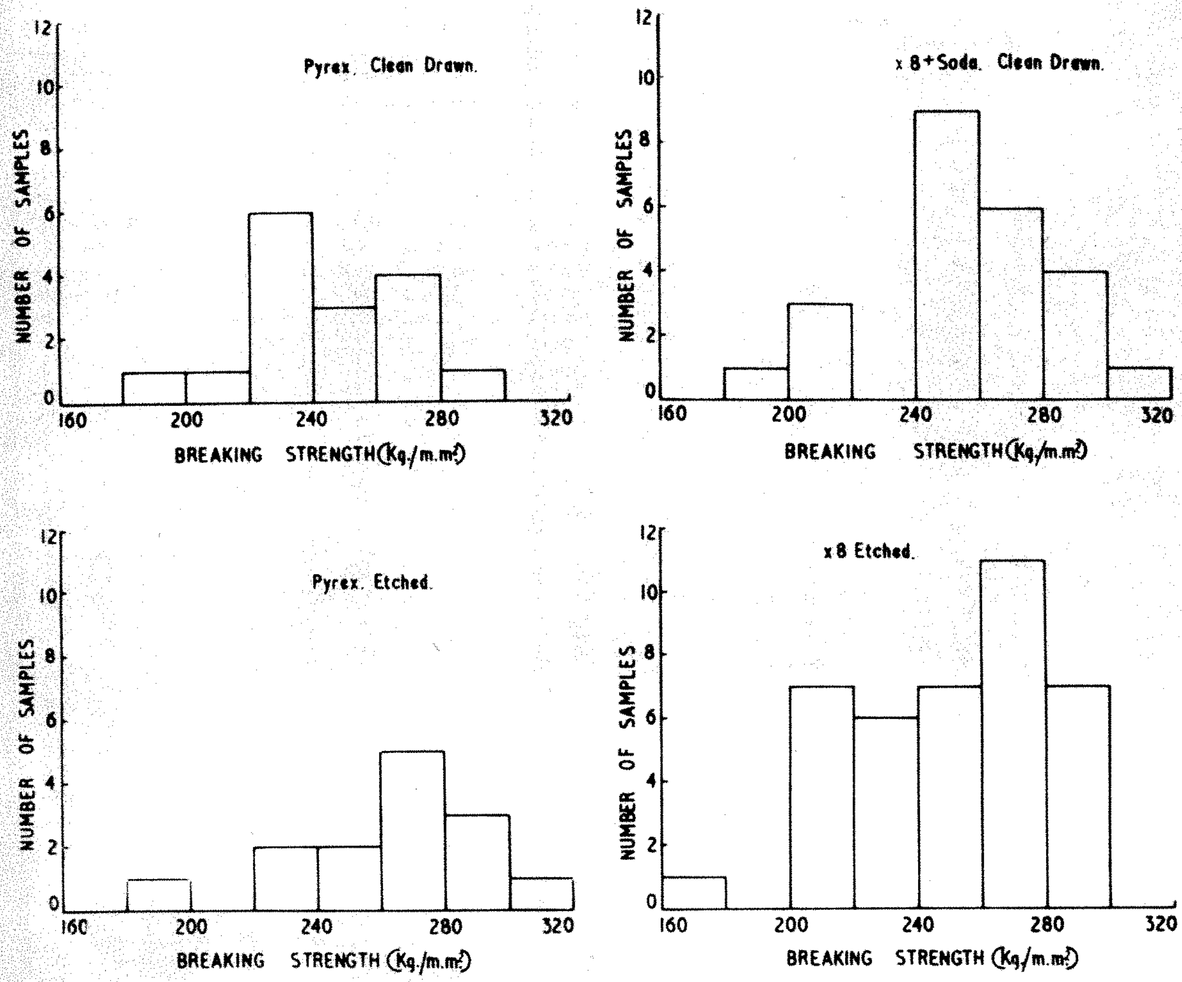

Fig. 1. Histograms illustrating the results obtained with 'Pyrex' and soda–lime–silica glasses

Table 1. SUMMARY OF RESULTS. IN THE SECOND COLUMN *C* INDICATES 'CLEAN DRAWN' SAMPLES AND *E* INDICATES ETCHED SAMPLES

Glass		Type of sample	No. of samples	Mean strength (kgm./mm.^{2})	Coefficient of variation
E-glass		*C*	24	343	7
Soda-Lime	×8	*C*	11	251	10
	×8	*E*	39	250	12
	Unknown	*C*	13	257	12
'Pyrex'		*C*	16	242	10
		E	14	265	11
Silica ('Vitreosil')		*C*	23	350	8
		E	11	361	11

were lower and the pits were more angular. Immersion for 5 min. in a 40 per cent solution did, however, yield appreciable attack and smooth shallow pits. It is the results for these samples which are quoted in Table 1.

The diameters of the broken ends of the specimens were measured with a micrometer eyepiece attached to the microscope. With the strong specimens considerable shattering occurred so that it was not possible to measure the diameter at the position of fracture, nor indeed to locate the origin of fracture. This feature did, however, enable us to eliminate from the results the comparatively few samples from the first series which failed at a 'defect', for when shattering did not occur it was possible to examine carefully both fracture surfaces and invariably to detect a visible defect at the edge of the mirror zone.

The breaking strength was assumed to be the maximum tensile stress at the surface and was computed using the simple formulæ for the stresses in a bent beam. Quite large deflexions do occur in these tests, but from the expressions given by Freeman[2] the errors involved in using the simple equations for the stresses do not exceed 2 per cent for most of the fibres and are less than 10 per cent for the finest.

The histograms of Fig. 1 illustrate the results obtained with 'Pyrex' glass and soda–lime–silica glasses, while all the results obtained in this preliminary series of experiments are summarized in Table 1. The results for etched *E*-glass fibres are not included because for this glass the reaction was complicated by the growth of layers of reaction product at the glass surface, and etching in hydrofluoric acid has not proved satisfactory for removing defects to leave a smooth surface.

Tests were made with the first type of sample at times varying from 4 min. to several days after production. No significant differences were observed in the strengths. Some of the etched samples were tested while still wet with distilled water from the rinsing bath; these also showed no significant difference in strength.

We conclude that the high and relatively consistent values of the breaking strength obtained in these experiments support quantitatively the suggestion made earlier that the visible defects are a major source of surface weakness[1]. The strengths are comparable with those obtained by Thomas[3] and Otto[4] for very fine fibres of *E*-glass, although still lower than that expected from theoretical estimates which assume a homogeneous structure for a glass.

The residual scatter in our results is larger than is to be expected from experimental errors, which is estimated to be less than ± 10 per cent for any individual determination. Since the magnitudes and the variabilities are similar for both the 'clean draw' and the etched samples of the several glasses, it seems unlikely that the scatter is due to undetected defects of known type in the surface. It has been established that the scatter in breaking strength for × 8 samples rises significantly when deliberate variations are made in the interval between removal of the rod from the flame and drawing the fibre. We think, however, that detailed discussion of these results and of the possible causes of the residual variability in strength should await the results from further experiments which are now in progress. These will enable samples to be produced under more carefully controlled conditions, to be annealed without the formation of fresh surface defects and to be tested so as to avoid any perturbing effects from the knife edges.

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Tortuosity in Porous Media

THE tortuosity, T, of a porous medium such as sandstone is a quantity that has been interpreted in various and sometimes conflicting ways, particularly its relation to porosity, φ, and formation factor, F (refs. 1–4). Tortuosity originally was understood to mean the square of the quotient average length of a flow-line divided by the net distance traversed; and formation factor was the electrical resistivity of a porous medium divided by the resistivity of the saturating liquid.

Wyllie and de Witte[5] have presented one of the several possible ways of showing that:

$$T = \varphi F \qquad (1)$$

is the only logical relation for models where the average volume of the pores is equal to their average length multiplied by their average cross-section. Equation (1) has been considered obvious by numerous investigators. However, it is not automatically correct for real systems, for which the value of T depends on the way the averages are taken. For example, in a randomly oriented set of straight uniform pores, the combination of their resistances in parallel shows that equation (1) holds with the harmonic mean of T and the arithmetical mean of the areas, while for a single pore of variable cross-section the reverse is approximately true.

Some rough calculations will serve to illustrate the chief characteristics. The geometry of 'flow-lines' accounts for both 'sinuosity' and 'convergence' of 'pores'[6]. Consider a conical segment expanding from radius a_1 to radius a_2, with straight flow-lines uniformly distributed. Let $r(x)$ be the distance of a flow-line from the axis; $r(0) = r_1$ and $r(L) = r_2$. Let θ be the angle between the flow-line and the axis; $\tan\ \theta = (r_2 - r_1)L$; $(a_2 - a_1)/L = \tan\Theta$, where Θ is the angle of the wall. For uniform distribution, $dr_2 = (a_2/a_1)dr_1$. Then:

$$\frac{1}{T} = \int_{\theta=0}^{\Theta} \cos^2\theta r dr \Big/ \int_{\theta=0}^{\Theta} r dr = \frac{(\log\ \sec.^2\Theta)}{\tan^2\Theta}$$

Now let δC be the total conductance of a sheath-shaped tube of flux between two conical surfaces : $\theta = \text{const.}$ and $\theta + \delta\theta = \text{const.}$

$$\frac{1}{\delta C} = \rho \int_0^L \frac{\mathrm{d}x/\cos\theta}{2\pi r.\cos\theta(\partial r/\partial\theta)\delta\theta}$$

where ρ is the fluid resistivity. The integration is performed by using the relation : $r = (x + a_1/\tan\Theta)\tan\theta$. The resulting expression is used to obtain the resistance of the whole segment :

$$R = 1/\int_{\theta=0}^{\Theta} \delta C = (\rho L \tan^2\Theta)/(\pi a_1 a_2 \log \sec^2\Theta)$$

Whence, for some arbitrary total area A :

$$T = (\pi a_1 a_2/A)F < \varphi F \qquad (2)$$

A measure of T independent of φ and F is possible, in principle, by such devices as transference lag[5] or transient diffusion[7,8]. If tortuosity is due mainly to pore 'sinuosity', and the arithmetical mean were measured, it might be that $T > \varphi F$, as the harmonic mean, appropriate for T in equation (1) for this case, is usually smaller. According to equation (2), the opposite would be expected when there are large variations of size in the pores.

Some authors have preferred to divest tortuosity of its geometrical significance and regard it simply as an impedance factor[9]. From this point of view, the tortuosity in the conical segment may be equated to φF, provided the corresponding approximation is made that the total resistance is the sum of the resistance of successive elementary segments in series. This familiar device amounts to assuming that flow-lines are geometrically parallel within each elementary segment. The result is :

$$T = (a_1^2 + a_1 a_2 + a_2^2)/3a_1 a_2 \qquad (3)$$

An analogous calculation for tortuosity in fluid-flow defines hydraulic resistance in terms of viscosity, η, as :

$$W = \frac{8\pi}{\eta} \int_0^L \mathrm{d}x/a^4$$

and a 'hydraulic formation factor', F_H, by :

$$W = \frac{F_H}{A} \int_0^L \frac{\mathrm{d}x}{\omega(x)}$$

where $\omega(x)$ is the average Poiseuille's-law mobility for the elementary segment at position x, given by :

$$\omega(x) = \int_0^a \omega(x,r) \frac{\mathrm{d}v}{\mathrm{d}r}\,\mathrm{d}r \Big/ \int_0^a \frac{\mathrm{d}v}{\mathrm{d}r}\,\mathrm{d}r$$

with

$$\omega(x,r) = (a^2 - r^2)/4\eta$$

$$v = \omega \mathrm{d}P/\mathrm{d}x$$

Integrating and combining :

$$F_H/A = (a_1^2 + a_1 a_2 + a_2^2)/3\pi a_1^2 a_2^2$$

which leads to :

$$\varphi F_H = [(a_1^2 + a_1 a_2 + a_2^2)/3a_1 a_2]^2 \qquad (4)$$

Equation (4) gives the square of the corresponding expression for electrical tortuosity in equation (3), but does not represent the geometrical tortuosity of the flow-lines. This provides a qualitative explanation for the fact that $(\varphi F)^2$ is the correlation function that has been derived from permeability experiments[1,10], while measured tortuosities have been close to φF in electrical migration and diffusion[5,8].

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Proton Relaxation in Crude Oils

IT was shown by Gutowsky, Ray, Rutledge and Unterberger[1] that crude oils exhibit electron resonance and by Poindexter[2] that the Overhauser effect is found in crude oils. The existence of the Overhauser effect depends on at least a part of the relaxation of the protons being due to the free electrons. Because the electron resonance and Overhauser effect results vary from sample to sample, it seemed of interest to make proton relaxation measurements on a number of samples of crude oil. The proton relaxation in crude oils is also of interest because of the introduction of proton precession instruments for underground measurements in oil wells[3].

Thermal relaxation, viscosity and density data were obtained for twelve Texas and two California crude oils. Temperatures were varied from 3° C. to 100° C., and field strengths varied from about 0·02 gauss to 7,000 gauss. Detailed relaxation curves were obtained by use of a low-field free-precession apparatus. Care was taken that the oils did not change composition by evaporation between measurement of viscosity and sealing of samples in 'Pyrex' bottles for relaxation measurements. Signal decay, or transverse relaxation, curves also were obtained in the Earth's field.

Fig. 1 shows that relaxation time is closely correlated with viscosity, despite a great range of composition of crude oils even in the limited selection of fourteen samples, and despite a probable variability of free-electron content. Apparently, only a fairly small fraction of the proton relaxation is due to the free electrons in most oils. There was no striking correlation between relaxation time and density. The relaxation times that are plotted in Fig. 1 are the reciprocals of the initial slopes of the relaxation curves, that is, the reciprocals of the average relaxation-rates. These results are for relaxation at 15 gauss. It was noted that the variation of T_1 with temperature for any one oil has about the same slope on a log–log plot as the general trend shown in Fig. 1.

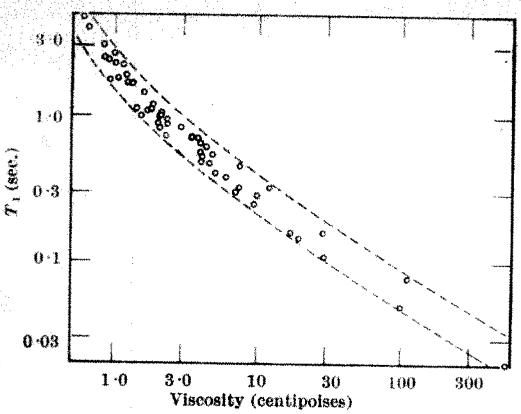

Fig. 1. Thermal relaxation times versus viscosity for 14 crude oils at various temperatures

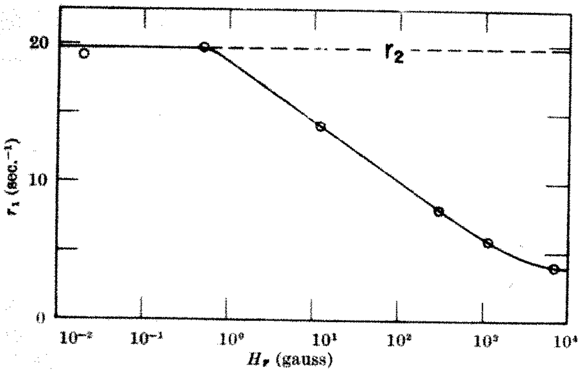

Fig. 2. Dependence of thermal relaxation-rate on magnetic field-strength for a crude oil sample

It was further noticed that there is no discontinuity at the 'pour point' for the oils exhibiting this phenomenon, although the macroscopic viscosity changes drastically as the temperature is lowered through this point.

Most of the crude oils showed a slight dependence on field for relaxation times, but only one sample exhibited strong dependence on field. The relaxation-rate (reciprocal of relaxation time) is shown as a function of field-strength in Fig. 2. At very low field-strengths, the thermal relaxation-rate is equal to the transverse relaxation-rate, which is indicated in Fig. 2 by the dashed line labelled r_2. Precession frequencies are presumably comparable to correlation frequencies in the range of field-strengths for which the relaxation-rate drops off with increasing field. If some of the relaxation is due to free electrons in the sample, it may well be that frequencies of electron precession are involved in the observed dependence on field.

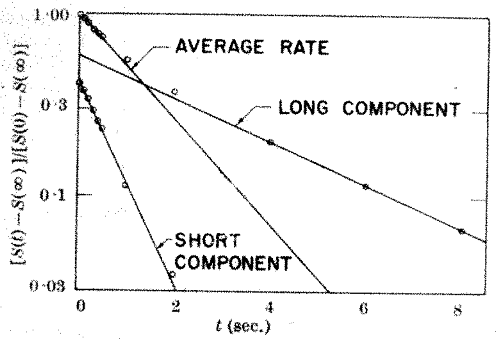

Fig. 3. Relaxation curve for a crude oil sample (different from sample represented in Fig. 2). S is the signal amplitude observed after allowing relaxation for a time t at the field for which relaxation data are desired

For all the oil samples the relaxation curves were noticeably non-exponential, and most of the curves can be represented within the accuracy of the data by the sum of two exponentials. In one of the samples there appeared to be an additional component of small amplitude and short relaxation time. The ratios of the pairs of relaxation times required to represent the relaxation curves varied from just over one to about five. The curves were carried out for relaxation by four powers of e in most cases. In many cases it was not possible to distinguish a curve composed of two distinct exponentials from a curve containing a distribution of relaxation-rates. Fig. 3 shows the relaxation curve for an oil for which the two relaxation-rates differ by a factor of nearly five. The curve labelled 'short component' shows the difference between the experimental points and the straight line labelled 'long component'. For this sample there appears to be two well-separated relaxation-rates.

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BIOPHYSICS

Direct-Current Conductivity of Textiles, Proteins and Other Polymers

There has emerged from a recent study of the electrical conductivity of dry cytochrome c (ref. 1) an understanding of two properties of the conductivity of a variety of natural and synthetic polymers:

(1) The two commonly observed conductivity–sorption relations (1) and (2) (ref. 2) are expressions of one mechanism; namely, conduction by ions in a medium of very low dielectric constant:

$$\log \sigma = A.M + \text{const. (small } M) \quad (1)$$

$$\log \sigma = B.\log M + \text{const. (intermediate } M) \quad (2)$$

where σ is conductivity; M, moisture content; and A and B, constants characteristic of the material. It is also observed that A is in the range 0·1–1 for a variety of materials, and B, 6–16.

(2) That the activation energies of conduction for dry amorphous substances should commonly exceed 25 kcal. (1·1 eV.) (ref. 1) is also understandable in terms of the same mechanism.

The theoretical approach adopted in ref. 1 was first used by King and Medley[3] in studying the conductivity–sorption relations of keratin and nylon, water and formic acid. They had previously shown that in moist keratin the current carriers were ions[4]. Treating the system by Bjerrum's theory of weak electrolytes, the polymer being considered the solvent, they were able, using their measurements on the dielectric constant of the system, to calculate curves of the fraction of unpaired ions versus the sorbent concentration, which reasonably reproduced important features of the conductivity-sorption curves. The semiquantitative agreement confirmed their expectation that variation of the number of charge carriers had a greater effect on the conductivity than the variation of carrier mobility, but that the latter was not negligible. King and Medley's approach merits wider consideration.

To demonstrate point 1, one displays the dependence of the fraction of free ions (α) on the dielectric constant (D), using the expression for the pairing equilibrium, which Fuoss[5] gives as:

$$(1 - \alpha)/c\alpha^2 = (4\pi N_0 \alpha^3/3000)\exp[|Z_1 Z_2| e^2/aDkT] \tag{3}$$

(c is total concentration in moles/litre; N_0, Avogadros' number; a, the distance between the centres of the ions in the pair; the other symbols have their usual meaning). Under the conditions of low dielectric constant that exist in polymers, the fraction of free ions is so small that the α in $1 - \alpha$ may be neglected, permitting one to write:

$$\ln \alpha = -cK_0/2 - Q/2aDT \tag{4}$$

where K_0 and Q represent the known constants. If one substitutes for D the approximation $D = D_0 + \beta M$, and writes α_0 as the value of α for $M = 0$, one obtains:

$$\ln(\alpha/\alpha_0) = (Q/2aD_0T)\cdot[\beta M/(D_0 + \beta M)] \tag{5}$$

For M small enough $\ln \alpha$ is linear in M; this is the basis of equation (1). The constant A is approximately $Q\beta/2aD_0^2T$. Calculated values of A agree with the observed closer than by an order of magnitude.

It is a property of rectangular hyperbolas such as (5) that they give a sigmoid curve when plotted versus $\ln M$ instead of M. In the region of the inflexion point an expression of the form of equation (2) holds. The original approximation used for D is not valid for the larger values of M, but a new linear approximation can be used which is valid in the appropriate range of M. The slope of $\ln \alpha$ in equation (2) is then

$$B = Q/8a[D(M_1) - \gamma M_1]T \tag{6}$$

The calculated values of B also match the observed to within an order of magnitude. Thus the two observed conductivity–sorption relations are manifestations of the same mechanism: conduction by ions in a medium of very low dielectric constant.

The activation energy of conduction will be the sum of the activation energy of the carrier mobility and the enthalpy change of the pairing equilibrium. The latter is the larger. Differentiating equation (4) one gets:

$$\Delta H_a = R.(Q/2aD).[1 + T(\mathrm{dln}a/\mathrm{d}T + \mathrm{dln}D/\mathrm{d}T)] \tag{7}$$

As the temperature coefficient of a will be $\sim 10^{-4}$, the term $T(\mathrm{dln}a/\mathrm{d}T)$ may be neglected; however, as $\mathrm{dln}D/\mathrm{d}T \cong 3 \times 10^{-3}$ at high frequencies[6], $T(\mathrm{dln}D/\mathrm{d}T) \cong 1$ in the range 0–100° C. The appropriate values of D are less than 6. Using these values and 2·8 Å. for a, the Na—Cl distance in sodium chloride[7], one obtains as a least value of the activation energy of conduction, $E_a = \Delta H_a = 20$ kcal./mole.

As the high-frequency dielectric constant is probably the correct one to use, corresponding to the rapid motion of the free ions, values of $D < 6$ will always be found. For example, carefully dried oxidized cytochrome c at 10 cycles/sec. and 100° C. has a dielectric constant of 3·1 (ref. 1). The large values of activation energy found in dry substances are therefore understandable in terms of an ionic theory of conduction.

The success of an ionic mechanism treated by the approach of King and Medley[3] in explaining two commonly observed properties of polymeric materials suggests that the conductivity of dry protein[8,9] is not unique in being electronic, but is another case of ionic conduction in polymeric substances. While a mechanism of electronic conductivity has been used with success to explain some conductivity properties of irradiated polymers[10], the conclusion reached above is that the dark conductivity is ionic, and calls for a treatment of the irradiated system from the ionic point of view.

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GEOMORPHOLOGY

Involutions and Ice-Wedges in Devon

RELICT periglacial phenomena have been recorded from south-west England for more than two hundred years. Reference has been made to head, clitter, rubble drift, loessic deposits and other manifestations of mechanical rock-weathering and ablation of debris under sub-arctic conditions[1], to geomorphological features which resemble actively developing tundra land-forms[2] and to fossil floras with arctic or alpine characters[3]. But so far as has been ascertained, no account has been given of phenomena which attest to the former existence of frozen ground (tjäle) in Devon.

An instructive illustration of involutions[4] is provided by a disused 'gravel' pit in decomposed, hydrothermally altered (tourmalinized) granite at 1,380 ft. O.D. on the gently sloping (< 2°) Cowsic/Blackbrook interfluve 1 mile west of Two Bridges, Dartmoor (*SX*/596752). The pit sections show up to 2·0 m. of decomposed granite (growan) and head on an uneven base from which open-jointed masses of sound rock project tor-like into the incoherent material. The highly irregular boundary between the brown, gravelly and gritty head of relatively fresh crystal and rock fragments and the underlying, paler growan with its characteristic flecks of white, decomposed felspars is emphasized, in places, by a prominent 0·5-cm. thick iron pan, a product of post-periglacial pedogenesis. But the involutions are not confined to an interdigitation of head and growan.

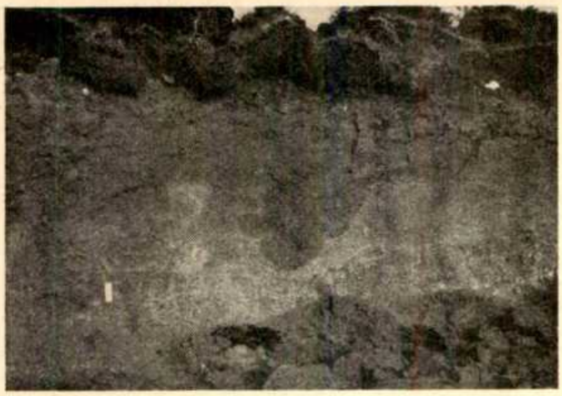

Fig. 1

Equally striking structures occur within the growan itself, which is disturbed to within 20 cm. of the base of the section (Fig. 1). A well-defined linear junction exists between the highly contorted growan characterized by a lineation of its altered felspars and the equally incoherent but undisturbed material in which the felspars have a random disposition as in adjacent blocks of sound granite. Thus it would appear that the lowest layer of the otherwise incompetent superficial mantle remained frozen while the overlying growan and head comprised an annual active layer or mollisol, and the involutions are expressions of the release of stresses set up during the re-freezing, from the surface downwards, of the seasonally thawed zone above the tjäle.

The example of ice-wedge pseudomorphs occurs in a pit at 450 ft. O.D. on the low western scarp of the Triassic Pebble Beds, 6 miles east of Cullompton (*ST*/066144). Unmistakable indications of frost-disturbance in the uppermost 1·0–2·0 m. of the pebble beds are provided by many exposures in the discontinuous escarpment, but it is reserved for the section in Fig. 2 to exhibit structures which are diagnostic of the former presence of the tjäle at such low elevations in Devon. The wedges penetrate up to 2·0 m. of the red, predominantly sandy, upper parts of the formation, the bedding of which is turned up sharply at their margins. They are filled with the same pale, yellowish sand that forms the bulk of the 60 cm. of head overlying the red rocks. It would seem that they were initiated as frost-cracks in frozen and thus impermeable sands and gravels, filled with melt-water from above, occupied by ice-wedges and afterwards filled with material washed in from the superjacent mollisol.

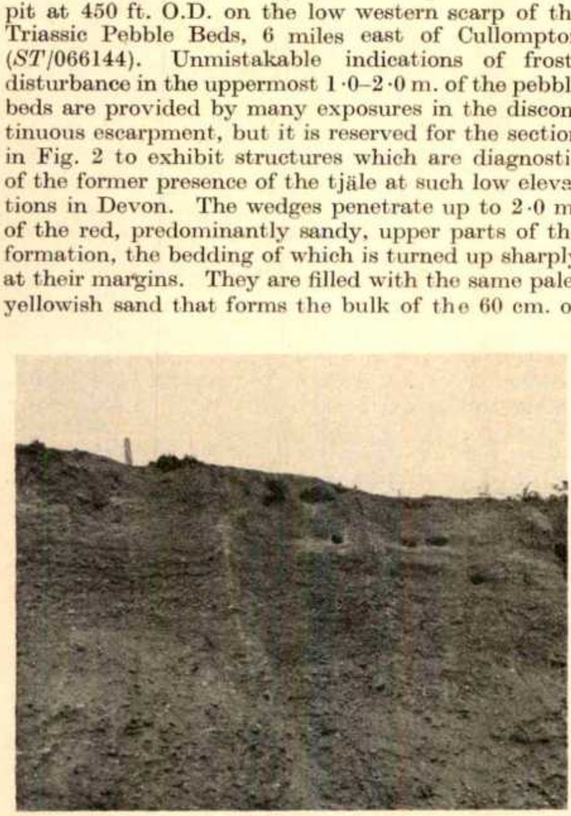

Fig. 2

A full account of these phenomena will be given elsewhere. But it may be noted that at least two distinct cryergic phases are represented at these and other sites. Thus we have evidence bearing not only on conditions during individual cold phases but also on the chronology of those Pleistocene phases during which the landscapes of south-west England experienced so profound a periglacial metamorphosis.

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CRYSTALLOGRAPHY

Crystals of Cellulose

RECENTLY several authors have described the growth of polymer crystals from solution[1-5]. These crystals are lamellar in structure, and it has been established that the chain molecules are oriented perpendicular to the plane of the layers. Since the average chain-length considerably exceeds the thickness of the lamellæ, it has been proposed that the chain molecules must be sharply folded in the crystals[3]. In comparison with the synthetic polymers on which these observations have been made, cellulose molecules are known to be relatively inflexible and should therefore be an interesting system for similar studies. This communication gives a brief preliminary account of the growth of crystals of cellulose triacetate and their conversion to crystals of cellulose.

The cellulose triacetate used had a degree of polymerization of about 300 and an acetyl value of 44·3 per cent. The crystals were grown from solution in nitromethane with concentrations in the range 0·05–3 per cent. Crystallization was effected by supercooling the solutions. In the case of the more dilute solutions where the nucleation rate appears to be rather low, crystallization was facilitated by adding suitable amounts of a non-solvent to the solution.

The crystals were visible under the optical microscope with phase contrast or incident illumination (Fig. 1). They were square in shape, and electron microscopy has shown them to be lamellar in structure. X-ray and birefringence measurements suggest that the chain molecules are oriented normal to the plane of the lamellæ.

Deacetylation of the crystals was carried out in 2 per cent potassium hydroxide solution in aqueous methanol (50 : 50 v/v) at room temperature for about 10 hr.[6]. Following saponification, the material was washed thoroughly with water and examined in the optical microscope. The external form of the crystals was not disturbed by the reaction, although considerable shrinkage (about 30 per cent) in the lateral

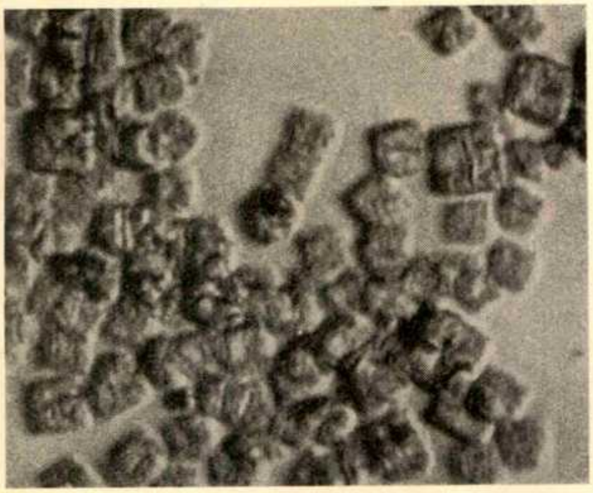

Fig. 1. Solution-grown crystals of cellulose triacetate (incident steep oblique illumination). (× 1,500)

dimensions had occurred. This contraction might possibly be accounted for on the basis of differences between the density and base molar weight of cellulose and the triacetate. The X-ray diagram (Fig. 2) of the deacetylated material shows it to have the structure of cellulose II, and indicates that the reaction had occurred throughout the lattice and not merely on the surfaces. Furthermore, optical birefringence indicates that the orientation of the chain molecules with respect to the layers was not disturbed by the deacetylation.

These observations suggest that, in spite of the relative stiffness of the molecules, folding similar to that observed in other polymers may also occur in cellulose.

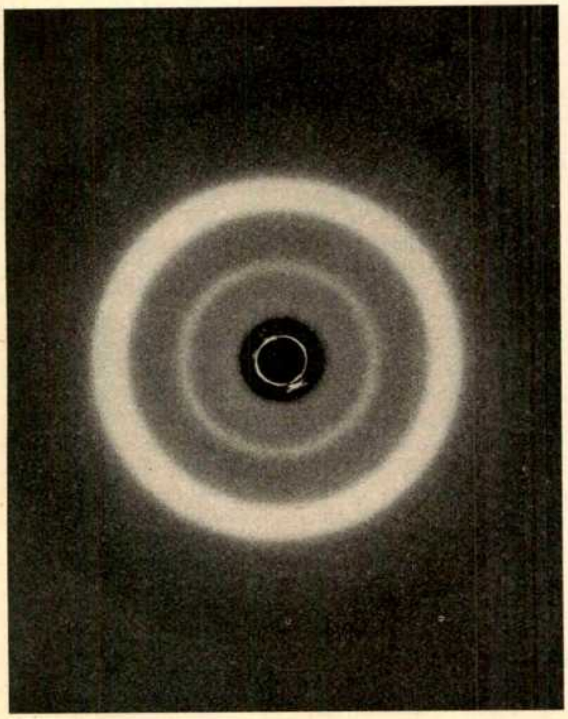

Fig. 2. X-ray diagram of deacetylated cellulose triacetate crystals

Detailed work on the morphology of these crystals is in progress and the results will be reported elsewhere.

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CHEMISTRY

Determination of Heats of Adsorption by Transient-Response Techniques

The classical equations of chromatography[1] have been applied to the determination of the heat of adsorption of a gas on a solid[2,3] by recognizing that the measured residence time, within a packed column, of a pulse of adsorbable gas relative to that of the carrier (or non-adsorbable component) may be related to an 'equilibrium constant' K_a by the equation:

$$\frac{t_c}{t_a} = \frac{1}{1 + K_a} \quad (1)$$

where t_a and t_c represent the retention times, respectively, of the active and carrier species. By assuming that $K_a \gg 1$ and utilizing the usual temperature functionality for K_a, Greene and Pust[2] show that the logarithm of the retention time of the adsorbing component is linearly related to the reciprocal of the absolute temperature. Thus the observed data on retention time versus temperature permit the evaluation of the adsorption heat, ΔH. Good agreement between values of ΔH determined by this technique and those obtained by others calorimetrically and/or isosterically was found by Greene and Pust for the adsorption of some low boiling gases on charcoal and for light hydrocarbons on alumina and silica gel at temperatures below 100° C. However, these authors correctly noted that no simple correspondence should exist between heats of adsorption derived from continuous-flow transient-response data (adsorption chromatography in their work) and those obtained by calorimetric methods, since the latter are functions of surface coverage.

The purpose of this communication is to extend insight into the phenomenon by reviewing the fundamental equations which govern the pulse dispersion and its residence time for various well-known adsorption equilibria.

I shall consider the behaviour of a concentration pulse passing through a tube packed with solid particles capable of reversibly adsorbing the pulsed component. Travelling at an average velocity $u = u_0/f$, where f is the fractional void volume of the packed section, the pulse will be dispersed by virtue of axial diffusion[4] characterized by a coefficient, D, and by reason of equilibrium adsorption. In terms of column-length, x, one may write[5]:

$$D\frac{\partial^2 c}{\partial x^2} - u\frac{\partial c}{\partial x} = \frac{\partial c}{\partial t} + \frac{(1-f)}{f}\frac{\partial n}{\partial t} \qquad (2)$$

where $\partial n/\partial t$ represents the transient on the solid phase and $\partial c/\partial t$ that change resulting in the gas phase due to the adsorption–desorption process. In terms of the fractional surface coverage, θ:

$$n = n_0\theta = g(K_e, c) \qquad (3)$$

where n_0 is concentration of total adsorption sites, K_e is an adsorption equilibrium constant, $Be^{-\Delta H/RT}$.

A number of cases follow once the form of the equilibrium law is specified.

Case 1. *Simple Linear Equilibrium.*

$$n = n_0\theta = n_0K_ec \qquad (4)$$

Thus:

$$\frac{\partial n}{\partial t} = n_0\frac{\partial\theta}{\partial t} = n_0K_e\frac{\partial c}{\partial t}$$

Equation 2 becomes:

$$D\frac{\partial^2 c}{\partial x^2} - u\frac{\partial c}{\partial x} = \frac{\partial c}{\partial t}\left[1 + \frac{(1-f)}{f}K_e\right] \qquad (5)$$

Let:

$$\beta = 1/\left[1 + \frac{(1-f)}{f}K_e\right] \text{ and } Z = x - \beta ut$$

then:

$$\beta D\frac{\partial^2 c}{\partial Z^2} = \frac{\partial c}{\partial t} \qquad (6)$$

Equation 6 is readily solved[6] for the boundary conditions commensurate with a pulse input of volume V, to yield:

$$c(x,t) = \frac{V}{fA\sqrt{\pi\beta Dt}}\, e^{-[x-\beta ut]^2/4\beta Dt} \qquad (7)$$

where c is expressed as volume of active component per unit volume of fluid and A is the column cross-sectional area.

The mean retention time measured in terms of the pulse maximum is obtained by setting the first derivative of equation 7 equal to zero.

$$t_a = \frac{D}{\beta u^2}\left(\sqrt{1 + (xu/D)^2} - 1\right) \qquad (8)$$

When $xu/D \gg 1$ (a condition realized in beds of more than several particle diameters of length[6]), equation 8 becomes:

$$t_a = \frac{1}{\beta}\left[\frac{x}{u} - \frac{D}{u^2}\right] = \frac{t_c}{\beta} \qquad (9)$$

where the term $[x/u - D/u^2]$ is the retention time, t_c, of the carrier, since axial diffusivity, D, has been shown[6,7] to be independent of the nature of the gas so long as the Reynolds number (D_pu/υ) is greater than 10. At Reynolds numbers less than 10, differences in molecular diffusivity, D_m, exert a negligible effect if $D_m/ux \leqslant 0{\cdot}1$.

In the terminology of chromatography, therefore, equation 9 is, in terms of equation 1:

$$\beta = \frac{t_c}{t_a} = \frac{1}{1 + \frac{(1-f)}{f}K_e} = \frac{1}{1 + K_a} \qquad (10)$$

It may be concluded that isothermal residence-time data permit a determination of K_e when the linear equilibrium prevails.

Case 2. *Freundlich Equilibrium.*

Where:

$$n = n_0\theta = n_0K_ec^n \qquad (11)$$

Equation 2 becomes a non-linear one:

$$D\frac{\partial^2 c}{\partial x^2} - u\frac{\partial c}{\partial x} = \frac{\partial c}{\partial t}\left[1 + \left(\frac{1-f}{f}\right)K_enc^{n-1}\right] \qquad (12)$$

Measurements of β in this circumstance are of doubtful significance since the derived 'equilibrium constant', K_a in equation 1, is obviously a function of concentration distributed in a gaussian or near-gaussian fashion.

Case 3. *Langmuir Equilibrium.*

$$n = n_0\theta = \frac{n_0K_ec}{(1 + K_ec)} \qquad (13)$$

Equation 2 again becomes non-linear, the apparent β being:

$$\beta = \frac{1}{\left[1 + \frac{(1-f)}{f}(1-\theta)^2K_e\right]} \qquad (14)$$

The direct determination of K_a by equation 1 in this instance leads to meaningless results, since surface coverage is a variable dependent on both concentration and temperature.

We may conclude that only in the circumstance where a simple linear equilibrium law prevails will a meaningful value of K_a be inferred from transient–response studies. A pseudo-linearity of the adsorption isotherm may indeed be achieved by an appropriate choice of pulse concentration. Alternatively, the relationship between residence time and pulse concentration at a given temperature may be extrapolated to zero concentration[3]. However, should the condition of linear equilibrium be realized, the significance of the derived adsorption heats demands closer examination.

If, following Boudart[8], the heat of adsorption is expressed as a function of temperature by the approximation:

$$Q = q_0 + aT \qquad (15)$$

then, even in the instance of linear equilibrium, the logarithm of retention time versus reciprocal temperature relation becomes, in terms of K_a derived from 1:

$$\ln K_a = \ln B + a/R + q_0/RT \qquad (16)$$

Thus the heat of adsorption so derived is the minimum (q_0) corresponding to high coverage. This point is of great importance in the study of chemisorption of reactants on solid catalysts as the value of chemisorption heat derived by transient techniques will have little significance in catalysis.

Referring to equation 14, it is apparent that, where Langmuir equilibrium prevails and surface coverage varies only slightly with concentration, derived values of K_a versus temperature may yield an unusually low value of the heat of adsorption since the term $(1 - \theta)^2$ varies with temperature in a manner which will decrease the slope of a plot of $\ln K_a$ versus $1/T$ plot to yield a value of $q < q_0$.

I wish to acknowledge the benefits derived from discussions of this subject with Profs. Paul Emmett

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Oxidation of Chromium(II) Amines by Water

AN interest in reactive reducing agents has led us to investigate aqueous solutions of chromium(II) containing excess of polyamines. We have observed that, in the absence of oxygen, these deep blue solutions (pH > 9) evolve hydrogen in accordance with the reaction:

$$2\mathrm{Cr(II)} + 2\mathrm{H_2O} \rightarrow 2\mathrm{Cr(III)} + \mathrm{H_2} + 2\mathrm{OH^-}$$

Although the reaction is quite slow when the amine is ethylenediamine or diethylenetriamine[1], with purified triethylenetetramine or *tris*(β-aminoethyl)amine[2] the reaction is sufficiently rapid to be studied conveniently. It is apparently autocatalytic with excess of the linear tetramine, and first-order in chromium(II) when the branched amine is present.

Hydrogen evolution from rapidly stirred solutions of chromium(II) perchlorate (0·02–0·05 M) in 0·3 M *tris*(β-aminoethyl)amine ($\mu = 1{\cdot}2$) was followed manometrically; it exhibited a strong dependence on pH. At 25°, the first-order rate constant was a maximum near pH 11 (4×10^{-4} sec.$^{-1}$) and lower by a factor of 10 at pH 9·5. No reaction was observable near pH 13. When all exchangeable hydrogen in reactants and solvent was replaced by deuterium, the rate constant was slightly decreased ($1{\cdot}0 \times 10^{-4}$ to $1{\cdot}3 \times 10^{-4}$ sec.$^{-1}$ at a glass electrode pH of 10·3). In isotopically normal media saturated with deuterium gas, the reaction did not yield measurable amounts of HD.

The other product of oxidation of chromium(II) by *tris*(β-aminoethyl)amine solutions, a violet chromium(III) complex, was subjected to chromatographic analysis on a column of 'Dowex 50 × 8' cation exchanger. Although it was slowly displaced as a single band by 0·01 M barium hydroxide, the complex could not be eluted by 0·01 N sodium hydroxide or, on the acid side, by N perchloric acid or 0·02 M lanthanum perchlorate in 0·01 N perchloric acid. This behaviour suggested that the oxidized chromium was bound as a binuclear complex carrying a charge of + 4 or more in acidic solution[3], until it was determined that *cis*-diaquo*bis*ethylenediamine cobalt(III) ion behaved similarly. The complex itself was isolated as the chloride by evaporation of the barium hydroxide eluate after precipitation of barium as the carbonate and subsequent acidification. This product had the same visible absorption spectrum as the reaction mixtures at complete reaction, maxima at 537 and 402 mμ, and gave chromium and nitrogen analyses indicating a ratio of chromium to *tris*(β-aminoethyl)amine of 1. The analytical results were not sufficiently precise to allow determination of a complete formula.

Although the observations detailed above do not suggest or rule out a mechanism for hydrogen evolution from aqueous chromium(II)–polyamine solutions, they are reported here since such evolution from homogeneous solution is uncommon.

A related observation, that chromium(II) added to a solution of *tris*(β-aminoethylamine) containing excess acrylamide is instantly oxidized to a complex different from that described, is still being investigated.

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Polarographic Behaviour of Chromium-Dipyridyl Complexes

IT has been shown previously that dipyridyl (dipy) complexes of cobalt and nickel are reduced at the dropping-mercury electrode with the formation of uni- and zero-valent complexes, respectively[1,2]. Further work led to the conclusion that iron(II)-dipyridyl complex is not reduced at the dropping mercury electrode and that the complex $\mathrm{Mn(dipy)_3^{2+}}$ is reduced with the formation of manganese(I) complex (unpublished results).

The complex $\mathrm{Cr(dipy)_3^{3+}}$, prepared by Hein and Herzog's method[3], is reduced at the dropping-mercury electrode in three waves, the ratio of which is 1 : 1 : 1. The comparison with the wave-height of the complex $\mathrm{Cr(NH_3)_6^{3+}}$ has shown that each of the waves of $\mathrm{Cr(dipy)_3^{3+}}$ corresponds to the acceptance of one electron for each particle of the complex. In aqueous 0·5 M sodium chloride solution, the slope of the first wave is identical with that of a reversible one-electron wave. The second wave, on the other hand, has a discontinuous character and the third one is drawn out (Fig. 1). In a solution of lithium chloride in 50 per cent ethanol, the first and second waves have the shape of a reversible one-electron wave, but the third one shows a sharp beginning. Furthermore, a very small adsorption wave is observed in ethanolic solutions almost at the same potential as that at which the discontinuous wave in aqueous solution appears (Fig. 1). The half-wave potentials (measured against the saturated calomel electrode) are summarized in Table 1.

Table 1. HALF-WAVE POTENTIALS OF THE WAVES OF $\mathrm{CR(DIPY)_3^{3+}}$ (V., 20° C.)

Solution	First wave	Second wave	Third wave
0·5 M Sodium chloride, water	−0·36	−0·73*	−1·38
0·5 M Lithium chloride, 50 per cent ethanol	−0·36	−0·71†, −0·895	−1·35*

* Discontinuous wave. † Adsorption wave.

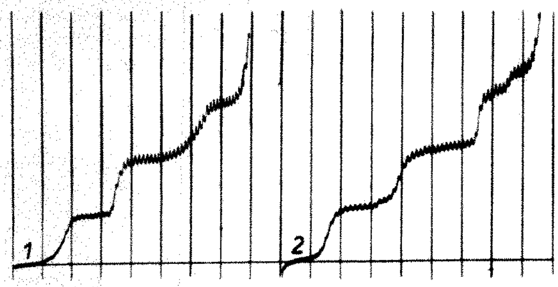

Fig. 1. The reduction of $Cr(dipy)_3^{3+}$. Curve 1, in 0·5 *M* sodium chloride (water). Curve 2, in 0·5 *M* lithium chloride, water–ethanol mixture, 1 : 1. Solution : 6 × 10^{-4} *M* $Cr(dipy)_3(ClO_4)_3$, from 0 V. (saturated calomel electrode), 200 mV. (absc.); nitrogen atm., *S* = 1 : 70

All three waves are diffusion currents. The addition of strong acids causes an increase of the second and third wave and the appearance of a kinetic, catalytic wave at negative potentials. This catalytic wave is formed even at very low hydrogen-ion concentrations, at which the increase of the second and third wave cannot be observed.

Using the Kalousek commutator, I have found that the product of the three-electron reduction is oxidized in three one-electron steps, the second and third being at the same potentials at which the reduction steps proceed. Similarly, the product of the two-electron reduction is reversibly oxidized in two steps and the product of the one-electron reduction in one step (Fig. 2). In experiments with the commutator in ethanol solution, the wave at − 0·71 V. is very pronounced, which is in agreement with its being an adsorption phenomenon.

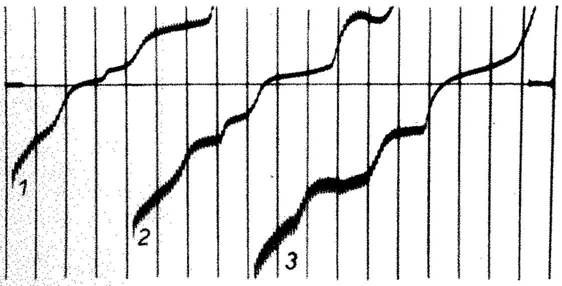

Fig. 2. Waves of complexes in lower oxidation states, generated by the commutator. Curve 1, wave of $Cr(dipy)_3^{2+}$, generated at 0·55 V. Curve 2, wave of $Cr(dipy)_3^{+}$, generated at −1·15 V. Curve 3, wave of $Cr(dipy)_3^{0}$, generated at −1·5 V. Solution: 6 × 10^{-4} *M* $Cr(dipy)_3(ClO_4)_3$, 0·5 *M* lithium chloride in ethanol-water mixture, 1 : 1 ; from 0 V. (saturated calomel electrode), 200 mV. (absc.) nitrogen atm., *S* = 1 : 150

The results described point to the conclusion that the complex $Cr(dipy)_3^{3+}$ is reduced in succession by one-electron steps, the final product being not metallic chromium but a complex of zero-valent chromium in solution which can be re-oxidized to the trivalent complex. The reversibility of the first reduction steps shows that the reduction proceeds without any change in the configuration of the complex, so that the following scheme can be written for the whole process :

$$\begin{array}{ccccccc} Cr(dipy)_3^{3+} & \rightleftharpoons & Cr(dipy)_3^{2+} & \rightleftharpoons & Cr(dipy)_3^{+} & \rightleftharpoons & Cr(dipy)_3^{0} \\ d_\varepsilon^3 & & d_\varepsilon^4 & & d_\varepsilon^5 & & d_\varepsilon^6 \end{array}$$

All complexes shown in the scheme have been prepared by Hein and Herzog[3], and their magnetic moments are in accordance with the electronic structure assigned by the letters d_ε.

The discontinuous shape of the waves is due to the formation of an insoluble reduction product. In aqueous solution the univalent and zero-valent complexes are insoluble, and hence the second wave is discontinuous. In ethanolic solution the univalent complex is soluble, but the solubility of the zero-valent complex is still very low, and accordingly the third wave has a discontinuous shape.

The first two reduction steps are reversible. The slight irreversibility of the third reduction step is probably due to the insolubility of the final product, the zero-valent complex.

The behaviour of the $Cr(dipy)_3^{3+}$ complex reported here is apparently the first known three-step reduction of a trivalent complex.

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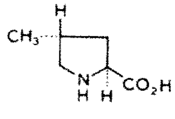
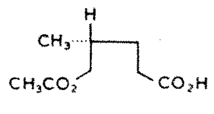
BIOCHEMISTRY

Absolute Configuration of 4-Methylproline from Perry

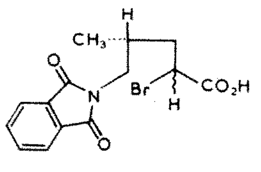
Two reports have appeared on the isolation of 4-methylproline from natural sources. Hulme and Arthington[1] isolated an amino-acid from apples, and tentatively assigned the structure of 4-methylproline to it as a result of elemental analysis, colour tests and chromatographic similarity to synthetic material. Later, two of us reported that hydrolysis of a peptide antibiotic yielded, *inter alia*, an amino-acid with the same chromatographic behaviour[2]. We have now been able to isolate 4-methylproline from perry (variety, Huffcap Seedling) by successive fractionation on 'Dowex-2' and 'Dowex-50' ion exchange resins. The crystalline amino-acid (8 mgm. from 5 litres of perry) has $[\alpha]_D$ *ca.* − 52° (*c.*, 0·3 in water), and is rigorously identified as *trans*-4-methyl-L-proline (I) by comparison with the optically active synthetic compound obtained as follows :



(I)



(II)



(III)

(+)-5-Acetoxy-4-methylpentanoic acid[3] (II) was converted by a mixture of sulphuric and hydrobromic acids to (−)-5-bromo-4-methylpentanoic acid, b.p. 114–18°/0·1 mm., the methyl ester of which, b.p. 96–100°/10 mm., condensed smoothly with potassium phthalimide to yield methyl (+)-4-methyl-

5-phthalimidopentanoate, m.p. 43–44°. Acidic hydrolysis of the methyl ester gave the acid, m.p. 73–74°, which was brominated with phosphorus and bromine to yield (+)-2-bromo-4-methyl-5-phthalimidopentanoic acid* (III), m.p. 141–42°. Vigorous acidic hydrolysis of the phthaloyl group and subsequent cyclization in aqueous sodium hydroxide furnished 4-methylproline*, m.p. 232–34° (dec.), $[\alpha]_D + 4$ to $+ 12°$ (in water, unchanged in 5 *N* hydrochloric acid). Separation of the *trans*-L-amino-acid was achieved through the solubility of its copper salt in absolute ethanol; decomposition of the copper salt with 'Dowex-50' (NH_4^+) cation exchange resin and recrystallization of the free amino-acid to constant optical rotation yielded *trans*-4-methyl-L-proline (I), m.p. 239–41° (dec.), $[\alpha]_D - 59°$ (*c.*, 0·3 in water), $[\alpha]_D - 32°$ (*c.*, 0·5 in 5 *N* hydrochloric acid). The positive shift in optical rotation on passing from neutral to acidic solutions establishes the L-configuration of the amino-acid[4]. Since (+)-5-acetoxy-4-methylpentanoic acid (II) has been converted to (−)-α-methylglutaric acid[5] of defined absolute configuration[6], the absolute configuration (I) of the synthetic 4-methylproline follows.

The infra-red spectra of the amino-acid from perry and the synthetic product (I) are virtually identical. Together with the correspondence in specific rotation, this establishes the absolute structure (I) for the natural amino-acid. It is noteworthy that the methylproline previously isolated from an antibiotic[2], although chromatographically indistinguishable from (I), has a quite different infra-red spectrum. In this connexion, isolation of the *cis*-4-methyl-D-proline diastereoisomer from the synthetic mixture is being attempted, and the results will be published elsewhere in the full account of this work.

As proline and glutamic acid are biogenetically related[7], it is likely that naturally occurring γ-methylglutamic acid[8] has the same configuration at $C_4(\gamma)$ as *trans*-4-methyl-L-proline, and hence may be accessible from the acetoxy-acid (II). Transformation of (II) into other methyl-substituted amino-acids is therefore being investigated.

We are grateful to Glaxo Laboratories, Ltd., for a generous supply of 5-acetoxy-4-methylpentanoic acid, and to the Department of Scientific and Industrial Research for a maintenance grant (to S. D.).

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* These materials consist of approximately equal quantities of the diastereoisomers with opposite configurations at the 2-position and the same configuration at the 4-position.

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Molecular Properties of Conjugated Proteins isolated from Exudates

THE protein constitution of exudates and transudates has been dealt with in a previous paper[1]. It was observed that the most peculiar property is the occurrence in the exudates of conjugated proteins with well-defined physical and physico-chemical characteristics. It has also been shown[2] that these conjugated proteins are the basis of the positivity of the Rivalta test.

In this communication the results of a preliminary investigation on the molecular properties of the conjugated proteins isolated from exudates will be reported.

These substances were isolated by dialysis of the exudate against acetate buffer at *p*H 4·5, ionic strength 0·1. A few hours afterwards a precipitate appeared at the bottom of the dialysis bag, and the precipitation was complete after 12 hr. The precipitate was collected, redissolved in phosphate buffer at *p*H 7, and again dialysed against acetate buffer at *p*H 4·5. At the end of the dialysis the precipitate was collected and analysed in the electrophoresis. At *p*H 7 two components were present: the first, about 60 per cent, had a mobility of $\mu = 6\cdot2 \times 10^{-5}$ cm.^{2} sec.$^{-1}$V.$^{-1}$, the second, about 40 per cent, with a mobility $\mu = 4\cdot5 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$, similar to that of serum-albumin. By means of a separation cell the two components were separately collected, and only the first one, that is, the largest and fastest, was shown to possess an exudative character with high positivity of the Rivalta test. The sedimentation pattern (Fig. 1) revealed that this component behaves in the ultracentrifuge as a monodisperse system, and its sedimentation coefficient, extrapolated to zero protein concentration, is $S^0_{20} = 3\cdot7 \times 10^{-13}$.

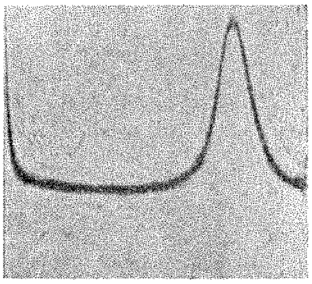

Fig. 1. 'Rivalta substance' isolated from serous exudate. Concentration: 1 per cent in phosphate buffer at *p*H 7. The photograph was taken 3·600 min. after reaching full speed of 59,780 r.p.m.

The diffusion constant calculated by the height/area method is $D^0_{20} = 2\cdot2 \times 10^{-7}$. Therefore, substituting in the Svedberg formula[3] the experimental values found for sedimentation, diffusion and partial specific volume ($\overline{V} = 0\cdot62$), the molecular weight was found to be $M = 108\cdot00$ and the frictional ratio $f/f^0 = 2$.

These results refer to a 'Rivalta substance' isolated from a serous exudate. In the case of other types of exudate, that is, hæmorrhagic or purulent, it has been observed that the molecular properties are somewhat different.

Research, now in progress, has provided clear evidence that several types of 'Rivalta substance' can be isolated, and, as previously stated, in any event there is a correlation between some biochemical aspects of the exudate and the type of inflammation.

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Effect of Polylysine on the Absorption Spectrum of Hæmin

THIS is a preliminary report on a 'polybase effect' consisting of a considerable change in the spectrum of hæmin on its interaction with polylysine in alkaline solution. Two different types of complex are formed with poly-L-lysine which may be associated with the folded and unfolded structure of the peptide. These simple hæmin–polypeptide complexes are considered to serve as models for iron–porphyrin-containing enzymes, and it is hoped that they will add to our knowledge of the prosthetic group-protein interaction and of the catalytic properties connected with it.

The polybase effect on the spectrum of hæmin is illustrated in Fig. 1. (The hæmin was 6×10^{-5} *M* in this and in all other experiments reported here.) Curve 1 represents part of the visible-range spectrum of hæmin at *p*H 11, while curve 2 was obtained under identical conditions except for the presence of 6×10^{-3} monomolar poly-L-lysine (molecular weight of the order 5,000). It is evident that the polybase induces a drastic spectral change on hæmin. (Also the Soret peak of the interaction product lies at 420 mμ as compared to 385 mμ for hæmin.) The addition of L-lysine monomer of the same monomolar concentration as the poly-L-lysine to hæmin had little effect on the hæmin spectrum between 750 and 230 mμ. This polybase effect can be observed visually: when viewed in measuring flasks of 20–25 mm. diameter, the hæmin and L-lysine–hæmin solutions appear brown, while the poly-L-lysine–hæmin solution is red in colour.

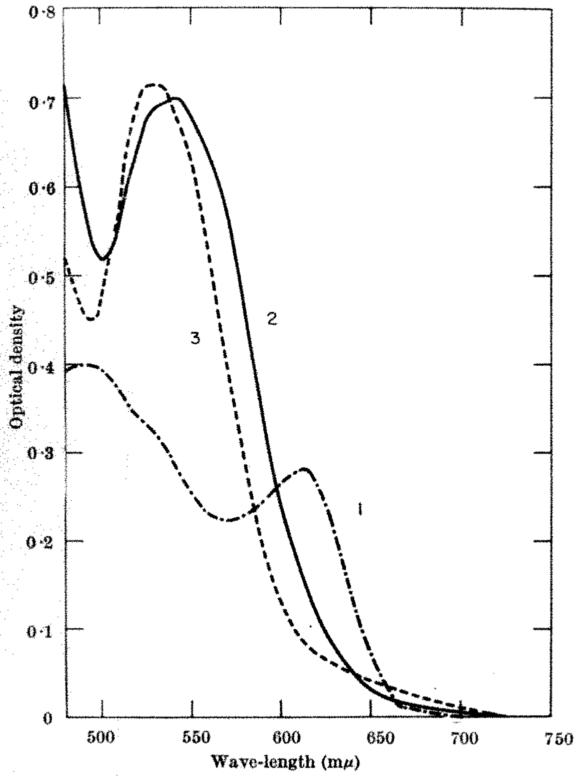

Fig. 1. Absorption spectra at *p*H 11. Curve 1, hæmin; curve 2, hæmin in the presence of poly-L-lysine; curve 3, ferricytochrome *c*

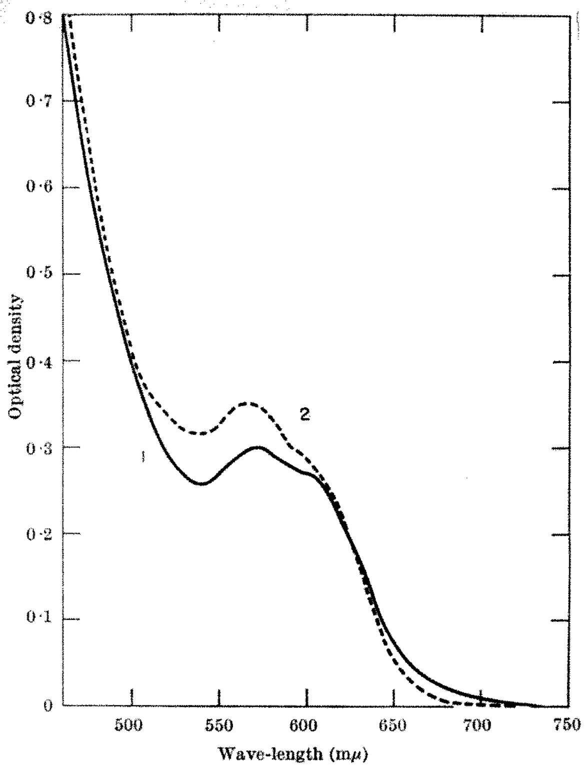

Fig. 2. Absorption spectra. Curve 1, poly-L-lysine : hæmin at *p*H 8–9 ; curve 2, poly-DL-lysine : hæmin at *p*H 11

On addition of hydrochloric acid to the red complex of poly-L-lysine with hæmin, the red colour changes to yellow-green at *p*H 9–10. Curve 1 of Fig. 2 is the measured absorption spectrum of this latter compound. Comparison with curve 2 in Fig. 1 confirms the drastic spectral change associated with this transition. (The Soret peak is at 388 mμ.) L-Lysine monomer of similar monomolar concentration again had no comparable effect on the spectrum of hæmin in the *p*H-region where the yellow-green complex is stable.

It has been shown[1] that poly-L-lysine in aqueous solution undergoes a pronounced change in optical rotation and other properties near *p*H 10. These changes have been correlated to a helix-coil transition occurring on decrease of *p*H. It would, therefore, seem reasonable to attribute the red complex described above to an interaction product of hæmin with some structurally ordered (helical) form of poly-L-lysine. Correspondingly, the yellow-green complex would be indicative of an unfolded peptide–hæmin interaction.

The following additional observations are believed to support these correlations: a sample of poly-DL-lysine of the same order of molecular weight and concentration as the poly-L-lysine used did not form the red colour with hæmin at *p*H 11, but instead showed the absorption characteristics of the yellow-green form which was obtained with poly-L-lysine at lower pH only (see curve 2, Fig. 2). This result is probably due to the formation of a random rather than a helical structure in the DL-copolymer. Moreover, a very low molecular weight poly-L-lysine of the order 600 at about the same monomolar concentration did not form a complex when mixed with hæmin at pH 11. Here, too, the absence of the red complex is probably due to the lack of suitable structure of the low molecular weight polypeptide. The folded form of the basic polypeptide apparently favours complete co-ordination to the hæmin by the basic groups of the side-chains.

The spectrum of ferricytochrome *c* from horse heart (about the same concentration in mgm./ml. as the poly-L-lysine) is shown for comparison in Fig. 1, curve 3. Though the spectra of the red poly-L-lysine–hæmin complex and that of ferricytochrome *c* are not identical—there appears to be a relative shift of about 10 mμ—it nevertheless seems possible to classify the cytochrome as a 'red complex' type. It would therefore be inferred that in ferricytochrome *c* the prosthetic group is bound to structurally ordered (helical) peptide over a wide range of pH-values (see refs. 2, 3 and 4).

It is obvious that the polylysine model compound described cannot be expected to conform in all respects to native ferricytochrome *c* and that it can only be considered to aid in our understanding of complex enzyme systems. We have found, incidentally, that poly-L-histidine forms a red hæmin complex similar to that of poly-L-lysine. Its region of stability is situated at lower pH than that of poly-L-lysine. In addition to a peak at 530–540 mμ there is a pronounced shoulder at 560 mμ. Poly-L-glutamic acid did not show any similar effect between pH 5 and 11·5.

A variety of physico-chemical methods has been employed during the past decades to determine the nature of the bonds involved in linking the prosthetic group to the protein in metal–porphyrin-containing enzymes, hæmoglobin and related compounds[4]. It is suggested that the differences in the spectra of the prosthetic group in these enzymes are conditioned not only by the different bond types but in some cases also by the secondary protein structure prevailing under the conditions examined. Thus the absorption spectrum of the prosthetic group–protein complex may serve as an indicator of the structural characteristics of the protein to which it is attached.

The effect of cyanide and similar small molecules acting as enzyme inhibitors on the spectrum of hæmin and of iron–porphyrin-containing enzymes is well known[4], and in many cases the spectra resemble those of the red complex obtained with poly-L-lysine and hæmin.

If the structural classification of the polylysine–hæmin complexes described above should prove to be correct, a simple and useful tool for identification and structural characterization of basic polypeptides and perhaps of other polybases may have been found which could yield valuable data down to low concentrations.

Further investigations by different physico-chemical methods including bivalent iron compounds and various polymers and copolymers are in progress. Details of the present work and studies of the catalytic properties of some of these compounds will be published in due course.

Thanks are due to the Department of Biophysics of the Weizmann Institute for the supply of various samples of polypeptides and for the determination of their molecular weight.

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Glutamic Acid Formation by Transoximase–Oximase Reaction

It has been previously reported that there exist in various organisms two enzymes, named transoximase[1] and oximase[2], which catalyse the transfer and reduction of the oximino group respectively. In view of the fact that glutamic acid plays an essential part in amino-acid synthesis, we have now studied glutamate formation through the combined action of these enzymes.

The enzyme solution was prepared from fresh liver of a young cockerel mainly according to the procedures for preparation *A* described in our earlier work[3] on oximase. However, the fractionation with acetone was carried out at − 10° C., and 5 gm. of the last precipitate were dissolved in 25 ml. of water at 0° C. After dialysing against cold running water for 3 hr., the solution was mixed with 8 ml. of *M*/5 phosphate buffer of pH 7·0 and centrifuged at 5,000 r.p.m. for 7 min. at 2° C. The supernatant contains the apoenzyme of oximase as well as of transoximase.

To measure the enzymatic actions, the following reaction mixtures were made (Table 1).

Table 1. Compositions of Reaction Mixtures

Test No.	0·2 *M* α-keto-glutaric acid (ml.)	0·2 *M* acet-oxime (ml.)	0·004 per cent pyridoxal phosphate (ml.)	0·2 *M* phosphate buffer, pH 7 (ml.)	Enzyme solution (ml.)	Water (ml.)
1	0·5	0·5	0·5	0·5	2·0	0
2	0·5	0·5	0	0·5	2·0	0·5
3	0·5	0	0·5	0·5	2·0	0·5
4	0·5	0	0	0·5	2·0	1·0
5	0	0·5	0·5	0·5	2·0	0·5
6	0·5	0·5	0·5	0·5	2·0 (*B*)*	0

* Heated at 100° C. for 10 min.

After incubating the mixtures at 33° C. for 45 min., 1 ml. of 0·12 per cent hydrogenated diphosphopyridine nucleotide solution, 0·6 ml. of 0·01 per cent flavin adenine dinucleotide solution and 1 ml. of 0·2 *M* phosphate buffer of pH 7·5 were added to each of them. The last mixtures were then maintained at 33° C. for 1 hr. After the reaction, the mixtures were heated at 100° C. for 10 min., mixed with 0·1 ml. of glacial acetic acid and centrifuged at 10,000 r.p.m. for 20 min. at 2° C. 5 ml. of the supernatant were added to 2 ml. of aqueous suspensions of 'Dowex-50'. After standing at 20° C. for 10 min., the suspension was decanted three times by adding water and then centrifuged at 3,000 r.p.m. for 5 min. at 25° C. The sediment was mixed with 1 ml. of 2 per cent ammonia solution. After centrifuging at 3,000 r.p.m. for 7 min. at 25° C., the supernatant was chromatographed.

The pyridine coenzyme used was prepared by hydrogenating with alcohol dehydrogenase from yeast; its solution, therefore, contains the dehydrogenase and alcohol which cause the rehydrogenation of the coenzyme dehydrogenated during the reaction. The 'Dowex' employed was treated beforehand with 4 *N* hydrochloric acid and *N* sodium hydroxide in the usual way, and further with 2 per cent ammonia solution and 1 per cent hydrochloric acid. After washing with water, the resin was suspended in water.

To estimate the amount of glutamic acid formed, 0·05 ml. of the ammoniacal supernatant was spotted in a width of 15 mm. on a Tōyō No. 2 filter-paper and developed with a butanol–acetic acid–water (4 : 1 : 2) solvent. At the same time, a guide spot was made with a standard glutamate solution. The filter paper was then sprayed with a 0·2 per cent ninhydrin solution. The coloured portion corresponding to R_F 0·32 was cut off, extracted with 4·5 ml. of 75 per cent alcohol, and the absorption ($-\log T$) at 570 mμ was measured with a Hitachi-electric spectrophotometer. The total quantity of glutamic acid produced in each reaction mixture was calculated from a standard curve established under our experimental conditions (Table 2).

Table 2. Amount of Glutamic Acid produced

Test No.	1	2	3	4	5	6
Glutamic acid (mgm.)	1·17	0·78	0·62	0·52	0	0

The results obtained show clearly that glutamic acid is formed from acetoxime and α-ketoglutaric acid by the action of transoximase and oximase. It was observed in the control that the amount of α-ketoglutaric oxime produced by spontaneous transoximation[4] is less than the lowest limit which can be detected by this chromatographic method.

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Replication of Deoxyribonucleic Acid in Non-dividing Bacteria

Deoxyribonucleic acid (DNA) is thought to be formed by replication. Meselson and Stahl[1] have used a density-gradient centrifugation technique to show that bacterial DNA is bipartite as was proposed by Watson and Crick[2] and that it reproduces in a semi-conservative fashion[3], each original sub-unit being found associated, on replication, with a newly formed sub-unit.

In the course of work on spontaneous mutation in non-dividing *Escherichia coli*, the possibility has been considered that errors may arise during DNA replication[4]. Although it is still a question whether such replication may account quantitatively for the mutations observed, it is clear that it does occur. Two systems of non-dividing *E. coli* have been employed in which there is a net increase of DNA but not of protein; they involve the starvation of histidineless auxotrophs for the amino-acid they require in the presence and absence of chloramphenicol.

E. coli 15 h^-(histidineless) were grown at 37° C. with shaking in a salts-glucose medium[5] to which $^{15}NH_4Cl$ was added as the sole source of nitrogen together with ^{14}N-L-histidine-hydrochloride (25 μgm. per ml.). Stationary phase cells which had ceased growth due to the exhaustion of glucose after 16-hr. incubation with shaking at 37° were harvested by centrifugation and washed twice with saline. These washed cells were transferred into nitrogen-14-salts-glucose medium with chloramphenicol (20 μgm. per ml.) but no histidine; aliquots were withdrawn at intervals up to 120 min. Estimations of DNA, RNA and protein were made by the diphenylamine, orcinol and Folin methods, respectively. As Table 1 shows, DNA increased by 79 per cent while RNA more than doubled; the change in the amount of protein was negligible and the number of viable cells remained constant.

Table 1. Synthesis of DNA, RNA and Protein of *E. coli* 15 h^- in a Histidine-Starvation Medium in the Presence of Chloramphenicol

Time of incubation (min.)	No. of viable cells (per ml.)	Relative amount per ml.		
		DNA	RNA	Protein
0	$3\cdot4 \times 10^8$	100	100	100
40	$3\cdot5 \times 10^8$	169	188	103
80	$3\cdot2 \times 10^8$	176	222	103
120	$3\cdot2 \times 10^8$	179	224	103

This DNA, formed in non-dividing cells without a concomitant net synthesis of protein, was investigated by the method of density-gradient centrifugation. The procedures for making the bacterial lysate by sodium dodecylsulphate and for centrifugation in a cæsium chloride solution were those described by Meselson and Stahl[1]. The ultra-violet absorption photographs of the equilibrated DNA (24 hr. at 44,770 r.p.m.) of the sample taken from the culture at 120 min. showed a well-defined ^{15}N–^{14}N hybrid DNA band together with a diminished ^{15}N–DNA band which had been observed as the sole band at zero time (Fig. 1).

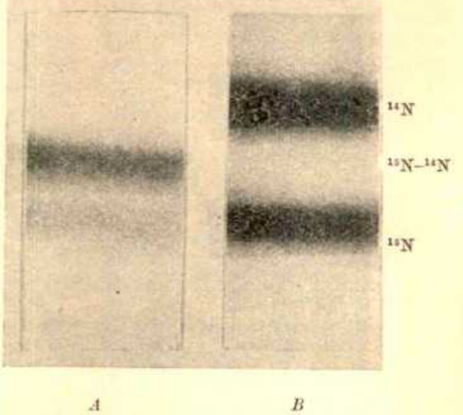

Fig. 1. Ultra-violet absorption photographs showing the DNA bands resulting from density-gradient centrifugation of: *A*, a lysate of *E. coli* 15 h^- labelled with nitrogen-15 taken after 120 min. incubation with chloramphenicol but no histidine in nitrogen-14 medium; *B*, a mixture of equal parts of lysates of *E. coli* 15 h^- labelled with nitrogen-15 and with nitrogen-14

A similar observation was made with *E. coli* 15 *t⁻h⁻*(thymine- and histidine-less). Cells labelled with nitrogen-15, grown in nitrogen-15-salts-glucose medium supplemented with ^{15}N-thymine (5 μgm. per ml., prepared from nitrogen-15-labelled cells of *E. coli* 15) and ^{14}N-L-histidine-hydrochloride (10 μgm. per ml.), were washed and transferred into nitrogen-14 medium with nitrogen-14-thymine, but no histidine, and incubated at 37° C. with shaking. After 120 min., DNA and RNA increased by 75 and 30 per cent, respectively, while the amount of protein and the viable cell number remained constant ($1{\cdot}5 \times 10^8$ per ml.)[6]. The sample taken after 120-min. incubation showed an intense ^{15}N–^{14}N hybrid band of DNA and a light ^{15}N–DNA band in the ultra-violet absorption photograph taken following density gradient centrifugation.

From these observations it is concluded that DNA formation involves replication in non-dividing, as well as in dividing, cells. This suggests the normality of the DNA formed without accompanying protein synthesis, which has been confirmed on biological grounds[7].

We are indebted to Prof. C. O. Beckmann of the Department of Chemistry, Columbia University, for his help with a Spinco *E* ultracentrifuge.

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Bovine Submaxillary Mucin

A CONVENIENT method for the isolation of bovine submaxillary mucin in a relatively undegraded and homogeneous state compared to earlier methods[1] has been developed in this Laboratory. Fresh glands (640 gm.) were ground and stirred mechanically with three changes of 500 ml. of cold water for a total of 54 hr. The pooled extracts were clarified by centrifuging at 26,360*g* for 30 min. and adjusted to *p*H 3·5. The resulting mucin clot was separated and dissolved in cold water by neutralization to 7·5, and lyophilized. The product was purified by solution in 500 ml. of cold 50 per cent (w/v) aqueous calcium chloride. After centrifugation at 26,360*g* for 30 min., the supernatant was fractionated with cold ethanol. The fraction separating at 60–75 per cent (v/v) ethanol was collected by centrifugation at 26,360*g* for 30 min., dissolved in water, dialysed against water and lyophilized. All procedures were carried out at 0–4° C. The yield was 0·64 and 2·5 per cent of the glands on fresh and dry weight basis, respectively. The recovery of sialic acid was 36 and 60 per cent of that in the extract and mucin clot, respectively. A repetition of the procedure did not alter the composition of the product. The lyophilized powder could be stored at 2–4° C. without change of its properties.

The mucin was soluble in water and gave clear, colourless, viscous solutions. The solutions (0·5 per cent) gave single peaks on electrophoresis and ultracentrifugation at *p*H 7·4 and 11·3. The electrophoretic mobility was $-6{\cdot}7 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$ in phosphate buffer, *p*H 7·4 and ionic strength 0·1. The sedimentation constant ($S_{20,w}$) was 2·6 at 0·5 per cent in the same buffer. No decrease in relative viscosity was observed when that of the product was compared with that of the extract at the same concentration of sialic acid. The intrinsic viscosity of the product in phosphate buffer, *p*H 7·4 and ionic strength 0·1, was 13·4 dl./gm. The mucin contained 9·2 per cent nitrogen, 28 per cent sialic acid, 24 per cent hexosamine (as N-acetylgalactosamine), 2 per cent neutral sugars (as D-galactose), and 37 per cent protein. D-Galactose and L-fucose were the only neutral sugars detected on paper chromatograms. Tyrosine and sulphur-containing amino-acids were not found on the chromatograms.

The mucin (1 gm. in 200 ml.) at *p*H 7·4 was incubated at 37° C. with 50 mgm. of crystalline trypsin. A reduction of the viscosity to that of the solvent occurred within a few minutes. After 24 hr. the digest was dialysed with only a slight loss in protein. The tryptic digestion was repeated and the mucin was recovered from the digest by precipitation with ethanol in the presence of 5 per cent (w/v) calcium chloride. After a reprecipitation the final product was dissolved in water, dialysed, and then lyophilized. The yield was 80 per cent. Its electrophoretic pattern showed a single peak at *p*H 11·3 with unchanged mobility. It contained 8·7 per cent nitrogen, 31 per cent sialic acid, 27 per cent hexosamine, 2·5 per cent neutral sugars, and 32 per cent protein. The relative viscosity of a 0·5 per cent solution in phosphate buffer, *p*H 7·4 and ionic strength 0·1, was 1·16. These results indicate that the protein occupies an integral part of the mucin molecule and also is associated with its rheological characteristics.

An aqueous solution of the undigested mucin (1 per cent) was heated at 100° C. with hydrochloric acid. Nearly all the sialic acid became dialysable after treatment at *p*H 2·5 for 30 min., whereas practically no hexosamine was liberated. After 120 min., the hexosamine lost by dialysis was about 25 per cent, and no loss of protein was observed.

These results are compatible with one of the earlier structures proposed by Gottschalk[2] of a protein core surrounded with N-acetylgalactosamine units with all sialic acid as external end-groups.

This work has been supported by grants from the U.S. Public Health Service, National Institutes of Health (*USPH A*–1303, *A*–216, *A*–1225) and from the U.S. Army Medical Research and Development Board, Dental Branch (*ASG MD*–773 and 774).

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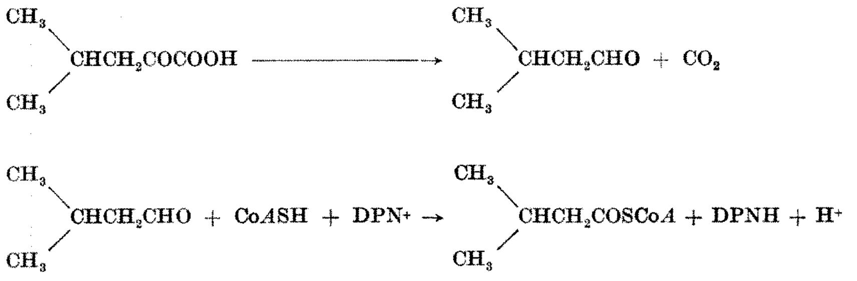
Degradative Pathway of L-Leucine in *Proteus vulgaris*

It was reported by Coon *et al.*[1–4] that in the metabolic pathway of L-leucine, *iso*valeryl coenzyme *A* was formed fromα -keto*iso*caproic acid, a deaminated product of leucine, in the presence of coenzyme *A* (Co*A*), etc. I postulate that this reaction involves the following two steps :



$$\underset{CH_3}{\overset{CH_3}{>}}CHCH_2COCOOH \longrightarrow \underset{CH_3}{\overset{CH_3}{>}}CHCH_2CHO + CO_2 \qquad (1)$$

$$\underset{CH_3}{\overset{CH_3}{>}}CHCH_2CHO + CoASH + DPN^+ \rightarrow \underset{CH_3}{\overset{CH_3}{>}}CHCH_2COSCoA + DPNH + H^+ \qquad (2)$$

In this communication it is reported that a cell-free extract which catalyses the reaction (1) was obtained from *Proteus vulgaris*.

Proteus vulgaris was cultivated at 37° C. for 18 hr. on peptone–broth agar slant. The cells were harvested, washed three times with de-ionized water, and suspended in 2×10^{-2} *M* phosphate buffer, *p*H 7·2. The washed cell suspension was disrupted by sonic oscillation in a Kubota vibrator, 10 kc./s., for 10 min. After centrifugation the supernatant was made 100 per cent saturated with respect to ammonium sulphate. The precipitate was dialysed against running water. In some cases, this dialysed protein solution was further fractionated with ammonium sulphate or cold acetone.

α-Keto*iso*caproic acid, used in the experiments, was prepared by extracting with acidic ether from the reaction mixture in which L-leucine was deaminated oxidatively by *P. vulgaris*. The melting point of the 2,4-dinitrophenylhydrazone of this keto-acid was 140–143° C. In this ether extract no other compound was detected by paper chromatography.

Carbon dioxide output was determined by the conventional Warburg method. The reaction mixture consisted of the following in a final volume of 2·5 ml. : $1{\cdot}67 \times 10^{-1}$ *M* acetate buffer at *p*H 5·0, 0·5 ml. ; 5×10^{-3} *M* magnesium sulphate, 0·25 ml. ; 2×10^{-1} *M* α-keto*iso*caproate, 0·125 ml. ; and enzyme, 1 mgm. protein. This reaction was carried out at 30° C. for 10 min.

The reaction of decarboxylation proceeded in nitrogen gas as well as in air ($Q_{CO_2}^{N_2}$ and $Q_{CO_2}^{Air}$ values were 1,158 and 1,207, respectively). The optimal *p*H was 5·0, and optimal substrate concentration was found to be 1×10^{-2} *M* ; half maximal activity was obtained with $1{\cdot}5 \times 10^{-3}$ *M*. Though it is not yet clear whether the reaction requires co-factors, coenzyme *A*, adenosine triphosphate, diphosphothiamine and Mg^{2+} were not required in the case of this crude enzyme.

Moreover, the reaction products were identified with paper chromatography. Three reaction systems were made as follows : (*a*) complete system (same as above experiments) ; (*b*) control system in which the enzyme was omitted ; and (*c*) control system in which the substrate was omitted. The volume of each reaction mixture was 10 ml. Immediately after incubation for 40 min. at 30° C., 0·5 ml. of mercuric acetate (5×10^{-1} *M*) was dropped in the reaction mixture. After the precipitate was discarded, 2,4-dinitrophenylhydrazine solution was added and stored at room temperature overnight. 2,4-Dinitrophenylhydrazone formed was washed thoroughly with hydrochloric acid, and dissolved with ether. The ether solution was used for paper chromatography. In experiment *c* no precipitate was formed. 2,4-Dinitrophenylhydrazone formed from experiments *a* and *b* and *iso*valeroaldehyde solution were developed with phenol (with 20 per cent water), *iso*amyl alcohol, and *n*-propyl alcohol as solvent, respectively. The results were represented in Table 1.

Table 1

Solvent	R_F value		
	Exp. *a*	Exp. *b*	Hydrazone of *iso*-valeroaldehyde
Phenol	1·00	0·81	1·00
*Iso*amyl alcohol	0·92	0·83	0·92
n-Propyl alcohol	0·91	0·82 ∼ 0·73	0·94

As shown in Table 1, one spot was obtained in experiment *a*, and the spot had the same R_F value of the hydrazone of *iso*valeroaldehyde. Furthermore, these spots were eluted from the paper with 15 per cent potassium hydroxide solution and were examined spectrophotometrically. It was concluded that *iso*valeroaldehyde was formed from this reaction system. The results reported in this communication and other experiments in which α-keto*iso*caproate was oxidized after vigorous decarboxylation, and reduced 2,6-dichlorophenolindophenol or methylene blue by this enzyme system in the presence of coenzyme *A* (and adenosine triphosphate and diphosphopyridine nucleotide), indicate that α-keto*iso*caproate was metabolized via *iso*valeroaldehyde (reaction (1)), and probably via reaction (2). Attempts at solving the latter reaction are in progress. Further details of this work will be published elsewhere.

I wish to thank Prof. S. Usami for his advice throughout the progress of this work.

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N-Ethyl-L-Asparagine : a New Amino-Acid Amide from *Ecballium*

MANY new amino- and imino-acids have been identified recently as plant constituents. A number of these newer compounds can be regarded as derivatives of either glutamic acid or glutamine, in which substituents occur on the carboxyl, amino or amido groups, or on the β- or γ-carbon atoms. The related compounds, aspartic acid and asparagine, are not known to form an analogous, extensive range of substituted derivatives. Now, however, *N*-ethylasparagine has been isolated from *Ecballium elaterium* (the squirting cucumber). Its relationship to theanine (II), isolated by Sakato[1] from tea leaves, is seen below :

$$(C_2H_5)HNOC.CH_2.CH(NH_2).CO_2H$$
N-Ethyl-L-β-asparagine (I)

$$(C_2H_5)HNOC.CH_2.CH_2.CH(NH_2).CO_2H$$
N-Ethyl-L-γ-glutamine (II)

The new amide was first recognized as an unidentified, reddish-brown coloured spot (*U*, Fig. 1) present on a ninhydrin-treated, two-dimensional chromatogram of a 75 per cent (v/v) ethanol extract of the aerial portions of *Ecballium*. The amide gives a colour reaction with ninhydrin that is identical with that produced by asparagine under the same conditions. The compound occupies a position close to that of proline on two-dimensional paper chromatograms developed in phenol-ammonia, followed by *n*-butanol–acetic acid–water. The R_F in the latter solvent is very sensitive to variations in temperature ; at 10–15°, the amide has an R_{Alanine} value of about 1·2, whereas at 25–30°, the R_{Alanine} value may be as low as 0·92.

The amide was isolated from 4·5 kgm. of *Ecballium* material. The extract was first fractionated on cation-exchange resin columns ('Zeokarb 215' and 'Dowex 50') and then further purified by chromatography on washed sheets of Whatman No. 3 *MM* filter-paper. About 1 gm. of crude isolate was obtained, which gave 300 mgm. of chromatographically pure amide after recrystallization from 75 per cent (v/v) ethanol. The isolate had the following elementary analysis : C, 45·2 per cent ; H, 7·7 per cent ; N, 17·6 per cent. $C_6H_{12}N_2O_3$ (*N*-ethylasparagine) requires : C, 45·0 per cent ; H, 7·6 per cent ; N, 17·5 per cent.

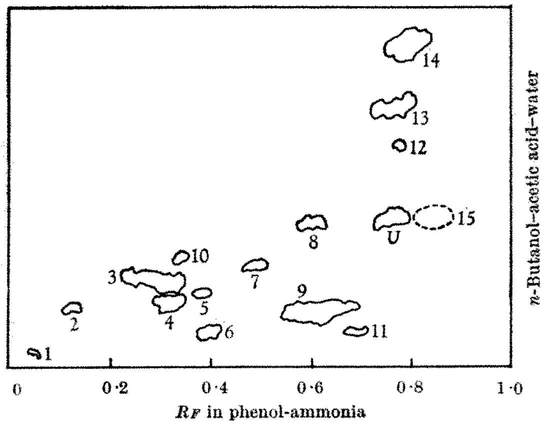

Fig. 1. Diagrammatic representation of a chromatogram prepared from an extract of *Ecballium*. The spots are : 1, cysteic acid ; 2, aspartic acid ; 3, glutamic acid ; 4, serine ; 5, glycine ; 6, asparagine ; 7, threonine ; 8, alanine ; 9, glutamine ; 10, α-aminoadipic acid ; 11, basic amino-acid ; 12, γ-aminobutyric acid ; 13, valine ; 14, leucines ; 15, position normally occupied by proline ; *U*, *N*-ethylasparagine

After hydrolysis with 6 *N* hydrochloric acid for 24 hr. at 100°, the amide was decomposed completely to yield aspartic acid and ethylamine hydrochloride. Assuming the structure (I), one mole of the isolate gave 0·96 mole of aspartic acid, and 0·98 mole of ethylamine. Aspartic acid was identified by comparison with an authentic sample by paper chromatography in four solvent systems (*n*-butanol–acetic acid–water mixture, 70 per cent (v/v) *n*-propanol, phenol-ammonia, and the upper phase of a mixture of ethyl acetate–pyridine–water, 2 : 1 : 2 parts by volume). Ethylamine, which was volatile in alkaline solution, was only chromatographed in the first two solvents and then as the hydrochloride.

The structure (I) was proved by comparison with synthetic material. After catalytic hydrogenation of N^2-benzyl-*N*-ethyl-DL-β-asparagine (kindly supplied by Dr. Liwschitz[2]), the main product, *N*-ethyl-DL-β-asparagine, was shown to be chromatographically identical with the isolated material in the above four solvent systems. The desired stereoisomer was prepared from L-aspartic acid, following the method of Hashizume[3]. β-Ethylaspartate, prepared by treating a suspension of aspartic acid in ethanol with dry hydrogen chloride at laboratory temperatures, was condensed with ethylamine, dissolved in a small quantity of ethanol, in a sealed tube at 100°. *N*-Ethyl-L-β-asparagine was separated from by-products by paper chromatography, and had the following analysis : C, 44·0 per cent ; H, 7·7 per cent ; N, 17·2 per cent.

The synthetic and isolated materials had the same melting point, 243° (decomp.), under identical conditions of very slow heating. The specific rotation in aqueous solution (*c*, 2·0 per cent) was also the same for both substances, $[\alpha]_D^{20°} - 5°$.

The natural and synthetic amides had infra-red spectra possessing identical absorption peaks (Fig. 2).

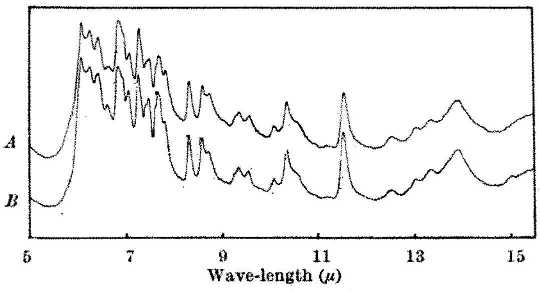

Fig. 2. The infra-red spectra of 'Nujol' mulls of *N*-ethyl-L-β-asparagine, (*A*) synthetic, (*B*) natural compound from *Ecballium*

The properties observed for the isolate confirm that it possesses structure (I) above, and so, more than 150 years after asparagine was first isolated from plant material[4], a naturally occurring derivative of the amide is now established.

Paper chromatographic evidence indicates that the amide is also present in the leaves of *Bryonia dioica* ; but it could not be detected in certain other Cucurbitaceae, including *Cucurbita pepo*, *Cucumis melo*, *Cucumis sativa* and *Echinocystis lobata*. An unknown spot (designated *U*131), observed in extracts of tulip leaves[5], moved to about the same position on paper chromatograms, and gave a similar reddish-brown colour after treatment with ninhydrin. Now it seems possible that this 'unknown' was *N*-ethylasparagine. It is interesting to recall that the concentrations of *U*131 and asparagine in tulip leaves, grown at different temperatures, showed a well-defined inverse correlation ; *U*131 was a more important constituent of leaves grown at 40° F., while asparagine was predominant in leaves grown at 70° F. Temperature could be affecting an equilibrium existing between asparagine and its *N*-ethyl derivative.

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PHYSIOLOGY

Release of Calcium-45 from Frog Nerves during Electrical Activity

Shanes and Bianchi[1] have demonstrated that stimulation of frog sartorius muscle accelerates the release of calcium-45 loaded on muscles, although Harris[2] had failed to observe this effect. Koketsu and Miyamoto[3] recently confirmed an increase in the output of calcium-45 during muscle activity. An important problem remains, however, whether acceleration of calcium output during activity is caused by the mechanism specific of the contractile system or actually is responsible for the electrical membrane phenomena. Hodgkin and Keynes[4] have reported that no change in calcium efflux can be detected during stimulation of squid axon, concluding that calcium movement during nervous activity is wholly inward. Furthermore, the 30-fold greater influx of calcium per impulse in muscle than in nerve despite the near equality of the resting influxes has been noted as consistent with a special role for calcium in contraction[5].

The experimental results reported here, however, clearly demonstrate that the release of calcium-45 loaded on nerve fibres apparently increases during electrical activity, suggesting that production of action potentials in muscles underlies the release of calcium at least in part. The discrepancy between the results observed with squid axon[4] and with the present material will be discussed at the end of this communication.

The present experiments were performed by using ventral- and dorsal-root nerves of bull-frog (*Rana catesbeiana*), which were dissected out together with the spinal ganglia and sciatic nerve plexus. These four preparations, isolated from a bull-frog, were tied together at the peripheral cut-end and suspended in a moist chamber. The root nerves were then immersed in Ringer's solution containing calcium-45 for 2 hr. at room temperature (24–26° C.). After washing the preparations for 20 sec. in Ringer, the time-course of the output of calcium-45 into normal Ringer was observed for 4 hr. During the procedure, the root nerves were successively immersed in a series of beakers containing 2 ml. Ringer every 20 min. Application of electrical stimulation (50/sec.) and recording of action potentials were made through electrodes placed on the sciatic nerves ; stimulation was applied for 40 min. during the 120–160 min. period after collection was started. The radioactivity of each collection, and the ashed root nerves, obtained at the end of the experiment, was measured. The rate coefficient curves[1] were constructed from these data.

Fig. 1 shows examples of the rate coefficient curves obtained from two experiments, to one of which stimulation was applied. The rate coefficient seems to settle within 120 min. to an almost constant value, so that it appears that the extracellular calcium-45 emerges within, at least, 120 min. and the slowly emerging calcium-45 is attributable to that coming from the axoplasma. The acceleration of output of calcium-45, induced by stimulation, therefore must be indicating an increase in the release of calcium from the nerve fibres. Presumably, the membrane calcium is removed during active depolarization by exchanging for external monovalent ions.

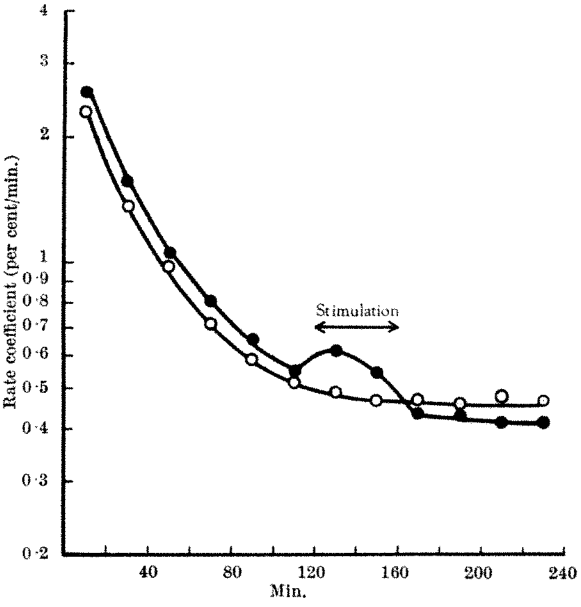

Fig. 1. Time-course of decline of rate coefficient of output of calcium-45 in Ringer. Two curves were obtained from two samples, to one of which stimulation was applied ; transitory rise in coefficients on application of stimulation is readily apparent

That the increased output of calcium-45 in squid axon was not observed[4] might have been due to technical factors. In most of these experiments, radiocalcium was injected into the axon for the purpose of excluding interference from the extracellular radioactive calcium and the efflux of this tracer calcium was observed. It seems that the efflux of intracellularly injected calcium-45 may be somewhat different from the calcium efflux observed in the present experiment, since (1) the method of application differs, and (2) the membrane calcium may easily exchange with the extracellularly applied calcium-45 but may not sufficiently with that applied intracellularly. It must also be kept in mind that the material differed from those used in the present experiment.

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Significance of Membrane Calcium in Calcium-free and Potassium-rich Media

Frog Ranvier's nodes immersed in potassium-rich media are capable of producing action potentials when the membrane is hyperpolarized by anodal currents[1]. This observation was confirmed in frog sartorius muscles by one of us, who found that action potentials were restored by anodal polarization in frog sartorius muscles which depolarized in calcium-free media[2]. The so-called 'hyperpolarizing response' was also observed in muscle fibres soaked in both potassium-rich and calcium-free media. This experimental evidence seems to suggest that removal of calcium from the membrane in these two solutions might be responsible for the loss of membrane excitability, as suggested in a previous communication[2]. The rate of output of calcium-45 loaded on muscles, therefore, was studied in both calcium-free and potassium-rich solutions.

Isolated frog sartorius muscle was soaked in Ringer containing calcium-45 for 2 hr. at room temperature. After washing the muscles for 20 sec. in Ringer's solution, the time course of the output of calcium-45 into normal Ringer and the test solutions was observed for 4 hr. During this procedure, the muscle was transferred successively into beakers containing 2 c.c. Ringer every 3–20 min. for 2 hr., and the same procedure was continued for another 2 hr. using beakers containing test solutions. The radioactivity in each beaker and muscle was measured, and the time course of the output was plotted (Fig. 1).

The output of calcium-45 markedly increased when the solution was changed from Ringer to either calcium-free (particularly with ethylenediamine tetraacetic acid[3]) or potassium-rich (5–30 m*M*) Ringer[4] (Fig. 1). Such increase was precipitous and transient, suggesting the membrane phenomenon. Furthermore, the increase of the outflux was observed without detectable muscle contraction. The total amount of calcium was slightly, but apparently, reduced in these muscles (Miyamoto and Koketsu, unpublished work). If such loss of calcium is responsible for inexcitability and also for depolarization, reduction of membrane potentials in these two solutions would be less pronounced when the release of calcium is prevented in some way. Actually, no appreciable depolarization was observed when cocaine (8×10^{-4} gm./c.c.) was added to the calcium-free Ringer in which the release of calcium-45 was apparently inhibited as shown in Fig. 1. Similar results were obtained by adding magnesium[3], strontium, cobalt, or nickel ions to the calcium-free solution. Depolarization in potassium-rich solution could also be prevented by increasing the external calcium or adding these divalent cations; the increased release of calcium-45 observed in potassium-rich solution was inhibited in these cases. Similar results were also obtained by adding cocaine (1×10^{-3} gm./c.c.) to the potassium-rich solution (Fig. 1). These experiments demonstrated clearly the close parallelism between the drop of resting potential and the output of calcium-45 in both calcium-free and potassium-rich solutions.

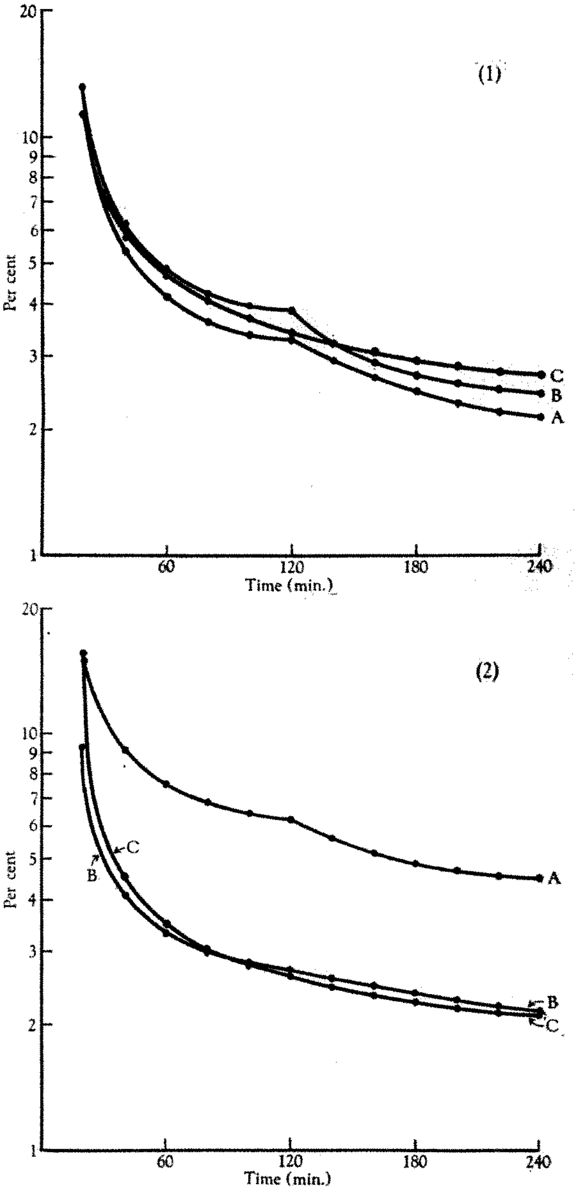

Fig. 1. Time-course of decline of calcium-45 content of individual muscles. Time-course during initial 120 min. was obtained in Ringer. After 120 min. bathing solutions were changed to test solutions: (1) *A*, calcium-free Ringer; *B*, calcium-free ethylenediamine tetraacetic acid (4 m*M*) Ringer; *C*, calcium-free Ringer containing cocaine (8×10^{-3} gm./c.c.). (2) *A*, potassium-rich (20 m*M*) Ringer; *B*, potassium-rich Ringer containing 50 m*M* calcium chloride; *C*, potassium-rich Ringer containing cocaine (1×10^{-3} gm./c.c.)

The observation[4] that the efflux of radiocalcium increases during muscle activity has been confirmed by us. The problem whether removal of calcium is consequential[4] or actually the cause of depolarization, in calcium-free and potassium-rich media or during stimulation, seems to be crucial.

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Rapid Reduction of Portal Blood Ammonia

Ammonia is a normal constituent of portal blood[1], the concentration in the mesenteric veins draining the distal portion of the small intestine, cæcum, and upper colon being 50 per cent higher than that in the peripheral circulation[2]. Most of the ammonia in the body is produced as a consequence of bacterial action on nitrogenous substrates within the intestine[2]. Investigations with isotopically labelled urea in several species of animals have revealed that urea is hydrolysed in the alimentary tract, even when administered parenterally[3], and, in man, 0·3 gm. of urea is hydrolysed per hour, and 15–30 per cent of the urea synthesized in normal subjects is continually being destroyed[4].

Attempts to lower the ammonia concentration of peripheral blood with antibacterial agents has yielded contradictory results. No change was found in the peripheral blood ammonia in dogs treated with oxytetracycline or neomycin[5,6]. In these experiments the initial values in peripheral blood were too low to reflect any change which might be due to the antibiotics.

Oxytetracycline, but not neomycin, lowered the blood ammonia concentration in the mesenteric vein of rabbits[7]. Dogs fed uncooked horsemeat for three days exhibited marked reductions in portal blood ammonia following medication with 6 gm. neomycin; sulphasuxidine and achromycin were much less effective[8]. This variation in response is believed due to the differences in flora found in herbivores and carnivores.

The present work is concerned with rapid reductions in portal blood ammonia following administration of an intravenous combiotic to hyperammonæmic dogs, together with evidence of an *in vivo* reduction of the intestinal bacterial population during the medication period.

Forty mongrel dogs, clinically free of disease and weighing 8–10 kgm., were fed 700 gm. raw horsemeat daily for 5 days. Medication was as follows: (1) medication in diet; (*a*) 50,000 units *K* penicillin + 25 mgm. streptomycin daily for 5 days; (2) medication after laparotomy; (*b*) 60 ml. saline, (*c*) 50,000 units *K* penicillin, (*d*) 25 mgm. streptomycin and (*e*) 50,000 units *K* penicillin and 25 mgm. streptomycin. Medications given after laparotomy were contained in 60 ml. saline, and infused into the cephalic vein at the rate of 2 ml. per min.

Laparotomies were performed under pentobarbital anæsthesia, and the caudal mesenteric vein leading from the descending colon exposed. Blood samples were withdrawn into heparinized syringes from the mesenteric and cephalic veins, prior to, and 2, 4 and 8 hr. after medication. Blood samples were obtained in a similar manner from dogs receiving antibiotics in their diet; in addition, samples were obtained at 24-hr. intervals for 5 days. Blood ammonia and urea nitrogen were determined by the microdiffusion methods of Conway[9].

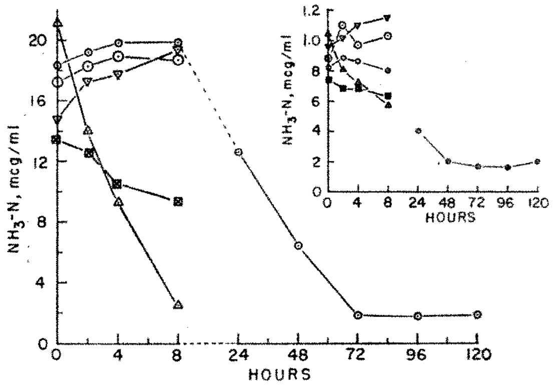

Fig. 1. Portal and peripheral blood ammonia 2, 4 and 8 hr. following intravenous medication with saline (○), streptomycin (⊠), penicillin (▽) and penicillin + streptomycin (△), or dietary supplementation with penicillin + streptomycin (○) over a 5-day interval. Each point on the graph represents the mean value for 8 dogs. Lower graph, portal blood ammonia; upper graph, peripheral blood ammonia

Fig. 1 shows the blood ammonia concentration, and Fig. 2 the blood urea nitrogen concentration, in portal blood 0, 2, 4 and 8 hr. after medication, and in the case of dogs with combiotic in the diet at 24, 48, 72, 96 and 120 hr. after medication. On each figure are superimposed the respective peripheral blood values obtained. The advantage of using penicillin and streptomycin together lies in their synergistic action when employed in combination[10,11]. Orally administered, non-absorbable antibiotics reduce the peripheral blood ammonia in hepatic coma[12] and cirrhosis of the liver[13] only after a 1–2-day interval. The need to lessen rapidly the ammonia load on the liver, thereby keeping the tricarboxylic acid-cycle functional, makes a slow-acting agent less than optimal. The results presented here demonstrate the value of using a combination of antibiotics which act synergistically and bring about a reduction in both the peripheral and portal blood ammonia within 4–8 hr. following administration.

A combination of penicillin and streptomycin reduces markedly the numbers of viable enterococci

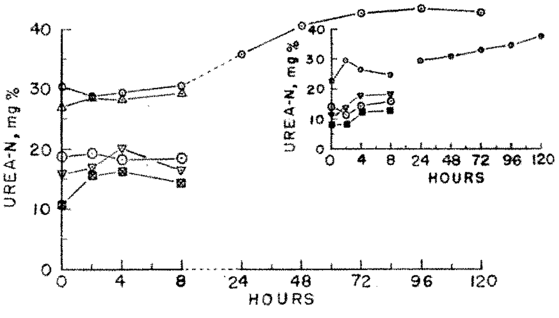

Fig. 2. Portal and peripheral blood urea nitrogen 2, 4 and 8 hr. following intravenous medication with saline (○), streptomycin (▽), penicillin (⊠) and penicillin + streptomycin (△), or dietary supplementation with penicillin + streptomycin (○). Each point on the graph represents the mean value for 8 dogs. Lower graph, portal blood urea nitrogen; upper graph, peripheral blood urea nitrogen

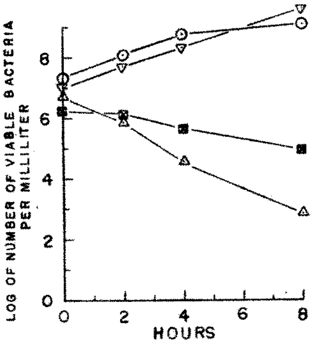

Fig. 3. Change in numbers of viable enterococci 2, 4 and 8 hr. following intravenous medication with saline (○), streptomycin (▽), penicillin (⊠), penicillin + streptomycin (△). Dilutions of fæcal aspirate were such that each ml. of broth contained the organisms from 1 gm. of fæces

in vitro[11]; however, this has not been demonstrated to occur rapidly *in vivo*. In the present work a sample of fæces was removed from the descending colon adjacent to the area from which the blood samples were obtained for cultures in McConkey's medium. Fig. 3 shows the changes in enterococci population at intervals corresponding to those when blood samples were obtained. It is readily seen that the reduction in blood ammonia and urea nitrogen parallels the reduction in number of viable intraluminal bacteria.

Part of this work was carried out while we were assigned to the U.S. Army Medical Research and Nutrition Laboratory, Denver, Colorado. We wish to acknowledge our indebtedness to Major R. O. Linder for performing the surgical operations.

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Effect of Phentolamine on Body Fat

SEVERAL recent publications have described an increase in the level of non-esterified fatty acids in the plasma of dogs or human beings after administration of certain adrenergic blocking agents. This effect has been observed in dogs injected with dibenamine[1] and dibenzyline[2] and in human beings treated with phentolamine[3].

Work in our Laboratories with phentolamine (phentolamine hydrochloride, 'Regitine^R' hydrochloride, kindly supplied by Dr. F. Yonkman, Ciba Pharmaceutical Products, Inc.), several other imidazoles and related structures has shown these drugs to have an action in reducing the rate of gain in weight of the *in situ* epididymal fat bodies of immature male rats. The drugs were administered subcutaneously twice daily for a 7-day treatment period to groups of six rats weighing 55–56 gm. Rats in a control group were injected with vehicle only. Change in body-weight and food consumption were determined daily. The fresh weight of the epididymal fat bodies was determined on the morning of the eighth day. Phentolamine administered at 4, 8 and 16 mgm./kgm. doses significantly decreased the fresh weight of the fat bodies by 16, 24 and 28 per cent, respectively ($P < 0\cdot05$), but did not significantly depress daily food consumption or gain in body-weight compared to control values. In other experiments daily food consumption was occasionally depressed.

Extension of this work led us to examine the effect of phentolamine on the extractable lipid of the carcass and adipose tissue. Three groups of five male rats weighing about 100 gm. at initiation of treatment were used. A control group was given carboxymethylcellulose vehicle (CMC) subcutaneously twice daily for a 2-week treatment period. A second group received 4 mgm./kgm. of phentolamine twice daily for 2 weeks. A third group was pair fed with the phentolamine group and received vehicle injections as in the first group. The day following the 2-week treatment period the rats were killed and the fresh weight and lipid content of the adipose tissue depots determined. Extractable lipid of the trimmed carcass was also measured. Lipid extractions were performed with chloroform/methanol (2 : 1). The results are shown in Table 1. The phentolamine-treated group showed significantly less total extractable lipid from carcass and epididymal fat bodies than either the control or pair-fed groups. The remaining intraperitoneal fat appeared to decrease in quantity but did not differ significantly from control values. Liver lipid content of phentolamine-treated rats did

Table 1. EFFECT OF PHENTOLAMINE ON CARCASS AND ADIPOSE TISSUE LIPIDS

Response	Treatment*		
	Full fed, CMC vehicle	Phentolamine (4 mgm./kgm.)	Pair fed, CMC vehicle
(*A*) Carcass			
Total extractable lipid (gm.)	6·2 ± 0·2†	4·3 ± 0·3‡	5·6 ± 0·7
(*B*) Epididymal fat			
fresh weight (mgm.)	651 ± 43	476 ± 8·3‡	654 ± 31
Total extractable lipid (mgm.)	420 ± 28	276 ± 5·5‡	394 ± 36
(*C*) Remaining intraperitoneal fat			
fresh weight (mgm.)	1,150 ± 61	930 ± 93	1,140 ± 24
Total extractable lipid (mgm.)	186 ± 12	154 ± 14	191 ± 35
(*D*) Total body fat§ (gm.)	6·79 ± 0·24	4·71 ± 0·22‡	6·14 ± 0·75

* Drug or vehicle given twice daily for two weeks.

† Mean ± *S.E.* for 5 rats.

‡ Significantly different from the full-fed control group ($P < 0\cdot05$).

§ Includes lipid of the carcass, epididymal fat bodies and intraperitoneal adipose tissue.

not differ significantly from control (results not shown).

The extractable lipids of the carcass were further characterized by separation into saponifiable and non-saponifiable fractions. The saponifiable fractions contained $80 \cdot 5 \pm 4 \cdot 0$, $56 \cdot 9 \pm 3 \cdot 3$ and $75 \cdot 2 \pm 2 \cdot 3$ per cent of the total lipids for the full fed, phentolamine-treated and pair-fed groups, respectively. The significant decrease in concentration of saponifiable lipids of the phentolamine group suggests that neutral triglycerides are most labile.

We have also observed in preliminary experiments (Fig. 1) that the plasma concentration of non-esterified fatty acids in groups of rats treated for 1 week with phentolamine was significantly increased. This increase was greatest on the day treatment was initiated and tended to fall to control-levels from the second to seventh days. In phentolamine-treated rats the level of non-esterified fatty acids in the plasma was significantly greater than control on days 0, 2 and 4. The gain in fresh weight of the epididymal fat bodies was most depressed during the first few days after which the rate of increase in weight paralleled that of control animals. The weight of the fat bodies was significantly ($P < 0 \cdot 05$) less than control on days 2, 4 and 7.

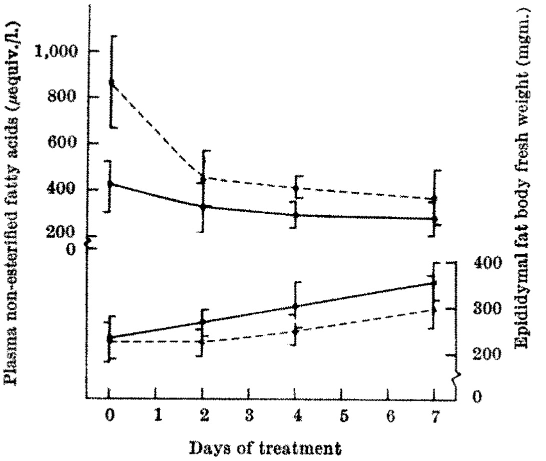

Fig. 1. Effect of phentolamine on plasma non-esterified fatty acids and the fresh weight of the epididymal fat bodies during 1 week of treatment. Determinations were made 2 hr. following the morning dose of drug on days 0, 2, 4 and 7 (6 rats per group). - - -, Phentolamine (8 mgm./kgm. twice daily); ——, control. [, 95 per cent confidence label

If phentolamine is acting to produce its effect on body-fat by some action on the autonomic nervous system, then it is possible that the observed results are secondary to catecholamine release either from the adrenal medulla or sympathetic nerve endings. Epinephrine does not appear to be implicated, however, since little or no change in blood glucose-levels has been observed by Klein and Bogdonoff[3] in humans or in our Laboratories (unpublished results) in rats. It is of interest to note that parenteral administration of phentolamine in man increased plasma norepinephrine- but not epinephrine-levels[4]. Norepinephrine increases plasma concentration of non-esterified fatty acids in man[5] and in dogs[6]. In addition, we have been successful in producing a depression of the fresh weight of the epididymal fat bodies with norepinephrine in oil using subcutaneous doses of 0·5–1·0 mgm./kgm. twice daily for 7 days. Experiments are in progress to determine the effect of phentolamine on the fresh weight of the epididymal fat bodies in adrenalectomized rats and on urinary catecholamine excretion.

This work was conducted in the Pharmacological Research Department.

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Liver and Depot Fatty Acids in Kwashiorkor

Two of the clinical features of the malnutritional state seen in tropical children and known as kwashiorkor are the marked increase of fat in the liver and the presence of depot fat in usual amounts. As the diet leading to this condition is poor in protein and fat, and contains relatively large amounts of carbohydrate, it was hoped that a study of the composition of the liver lipid might lead to a clearer understanding of the origin of this lipid. Earlier work[1] on the phospholipid, non-saponifiable and fatty acid fractions of the liver lipid in kwashiorkor showed that their proportions and degree of unsaturation were not the same as in excess liver lipid found in children who were apparently well nourished but suffering from other diseases.

To find out if the fatty acid composition of the liver lipid was also different in kwashiorkor from that in fatty livers due to other causes (control series) the lipid from post-mortem portions of liver from children aged 1–4 years was extracted with petroleum ether and the acids obtained by hydrolysis of the lipid were examined by gas chromatography. The results (mean values and *S.E.*) can be seen in Table 1, where comparable results from a similar examination of the depot fat can also be seen.

The findings are as follows: (1) There is a greater proportion of C_{18} unsaturated ($C_{18} : \Delta$) fatty acids in liver lipid in kwashiorkor. (2) There is no correlation between the proportion of $C_{18} : \Delta$ acids in the liver and the amount (gm./100 gm. liver) of liver lipid. (3) The kwashiorkor depot fat C_{14} and C_{16} acids are reduced and $C_{18} : \Delta$ fatty acids are increased. (4) No correlation exists between the portions of the various fatty acids in the depot fat and the amount of liver lipid. (5) In controls the proportions of fatty acids in the liver differ from those in the depot fat. (6) In kwashiorkor the liver lipid fatty acid com-

Table 1

	C_{12}	C_{14}	C_{16}	C_{18}: Δ	C_{18} : O	No. examined
Lipid from :						
Liver Kwashiorkor	Trace	3·8 ± 0·78	34·8 ± 3·14	[53·6] ± 4·69	7·8 ± 1·57	5
Liver Control	Trace	4·9 ± 0·10	39·7 ± 2·26	43·5 ± 1·66	11·9 ± 1·28	11
Depot Kwashiorkor	2·9 ± 1·11	[8·3] ± 2·61	[30·0] ± 5·57	[51·6] ± 4·79	7·3 ± 0·81	4
Depot Control	7·9 ± 1·67	15·8 ± 1·73	44·9 ± 2·87	26·4 ± 2·80	5·1 ± 0·98	7

(Figures in square brackets indicate that when compared with control $P = < 0·05$.)

position is very similar to that in the depots. (7) The proportions of fatty acids in the depot fat in kwashiorkor are very similar to those described in the European adult[2,3].

From these preliminary observations it seems that the fatty acid composition of the liver lipid in kwashiorkor is different from that in other types of fatty liver found in comparable children. Also in kwashiorkor the proportions of the fatty acids in the liver fat are very similar to those in the depot fat, and these in turn are very similar to values given for depot fat of normal European adults.

One of us (I. M.) wishes to thank the Ciba Foundation and Institut National d'Hygiène, Paris, for a grant enabling him to work in French West Africa.

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Relations of Intensity of Photosynthesis and Appearance of *Piricularia* oryzae Cav. on the Rice Plant

Piricularia oryzae Cav. appears in the rice fields of Hungary in certain years, when a considerably decreased yield often occurs. However, appearance of the fungus did not cause a decrease of yield in every case. It was noted that the crop in some cases was not affected at all with the plants infected by *Piricularia*, whereas in other cases there was total damage in absence of the fungus. As a result of several years of experiment it may be stated that the fungus which, in the opinion of some workers, is the cause of browning disease ('brusone') appears only on certain soil-types. Results obtained so far of the investigation of browning disease suggest that the presence of fungi is rather a consequence of the effects and conditions of unfavourable soil and weather on the plant than connected with the browning disease proper.

Research work on the cause of browning disease led to the view that injury resulted from hydrogen sulphide formed in the water-logged soil. Hydrogen sulphide inhibits respiration and uptake of water and nutrients by the plant[1]. Since the hydrogen sulphide is removed by oxygen, we considered the problem to be practically cleared up. At the same time both the disease and the fungus were absent in years when solar radiation was abundant and the photosynthetic processes were not impeded[2].

Further experiments were carried out to elucidate the relation between browning disease, the appearance of *Piricularia oryzae* and the intensity of photosynthesis. In our experiments we used the well-known Dunghan Shali variety, which is susceptible to browning disease. As it is well known that the amount of photosynthesis is different for light of different colours, plants in the same developmental stage were exposed to different colour effects. For this purpose four iron stands (130 cm. × 130 cm. × 200 cm.) were made and covered—except for the bottom—with black, white, red, and blue linen. These frames were placed under identical conditions over plants in various phases (tillering, stem elongating, blossoming) of development each for 6 days and the changes occurring in the covered plants were observed. Before the elongation of the stem, no fungal infection could be found in any of the plants.

Following elongation of the stem, the following observations were noted : Plants under a black cover died in every case. Those under a blue cover became thin and developed no further. Under red they elongated, while the leaves of the plants under white were covered with *Piricularia oryzae*. The leaves of the plants under a red cover showed no more fungal spots than those of control plants, where they were insignificant. After removal of the white cover the fungus-spotted plants produced under it continued development and gave crop.

As regards redox potential of the soil and oxygen content of the soil water, the most favourable conditions were under the red cover.

From these observations we conclude that the synthetic processes of the rice plant under the white cover are, to a certain extent, inhibited. In the leaves, as a result of disturbed physiological processes, free amino-acids, mainly glutamin and asparagin, accumulated and rendered possible the appearance of the fungus. On the other hand, under the red cover, the undisturbed vital process of the plants developing in the abundant red light prevents the fungus from becoming established. In these experiments, fungal infection could occur only after the elongation of the stem, that is, in the generative phase when the quantity of oxygen carried to the roots decreased significantly.

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HÆMATOLOGY

Relationships between Blood Groups and the Fat Percentage of the Milk in Cattle

CATTLE show pronounced polymorphism with regard to blood groups. No less than eleven loci governing blood groups have been detected, and some of these carry very extensive numbers of multiple alleles.

Recent work[1–4] indicates the existence of relationships between some blood-group genes and characters of economic importance in cattle. Mitscherlich *et al.*[1,4] presented evidence for a negative influence of the M gene on total milk yield. I compared[2] the fat percentage of the milk, total fat yield and the heart-girth measurements of paternal half-sibs having and lacking certain blood-group genes belonging to eight different genetic systems. Statistically significant associations were found particularly between some blood genes and fat percentage; the latter trait having the highest heritability of the three production characters studied. Especially one of the multiple alleles at the B locus, namely, $B^{BO_1YD'}$, seemed to exercise a strongly positive influence on the fat percentage. Heifers carrying this allele had, on the average, a fat percentage which was 0·19 unit higher than that of their paternal half-sibs lacking this allele. Similarly the J gene had an increasing effect, while the L gene showed a negative influence on the fat percentage. A seemingly pleiotropic effect of some B alleles on the fat percentage was also reported by Neimann-Sørensen[3].

If a large number of tests are performed, statistically significant effects may be indicated even if no real biological association exists. The associations between the $B^{BO_1YD'}$, J and L blood-group genes and fat percentage found in my earlier paper have therefore been re-investigated on independent material.

Since 1955 the blood groups of all bulls used for artificial insemination in Sweden are tested. Records are now available of the daughters' fat percentage of several of these during the first lactation (305 days) and the deviation of each daughter-group from the contemporary herd averages with regard to fat percentage. These records (compiled by the bureau for progeny tests, N.A.T.) furnished material for the present study. Only those bulls were included the blood of which was tested before anything was known about the production of their progeny. For each of the blood groups studied, the bulls were divided into two classes, namely, those having and those lacking a particular blood group. For the Swedish Red and White breed (SRB) 205 bulls were studied, each having between 10 and 41 daughters (average 16·7), and in the Swedish Friesian (SLB), 77 bulls with 10–37 daughters each were analysed. The influence of the blood groups of the sires on the butter-fat test of their progeny is summarized in Table 1. For comparison the results obtained in previous work[2] on the effects of blood group genes within progeny groups are given in the right column of Table 1. The allele $B^{BO_1YD'}$ exercised a strongly positive influence on fat percentage in both the SRB and SLB breeds. Thus the fat percentage in daughter-groups from sires carrying $B^{BO_1YD'}$ exceeded that of the daughters from the remaining sires by approximately 0·10 unit. Most of the sires carrying $B^{BO_1YD'}$ were heterozygous. Slightly more than half their daughters may therefore be expected to have $B^{BO_1YD'}$. The total increasing effect of this allele on the fat percentage can therefore be estimated at about 0·20 unit. It may be recalled that in previous work carried out within progeny groups, the heifers having $B^{BO_1YD'}$ produced milk with 0·19 unit higher fat percentage than those lacking this allele.

In both the breeds the butter fat test of daughters from J-positive sires exceeded that of the offspring from sires lacking J with approximately 0·04 unit. The difference was not statistically significant within any of the breeds, but when the data on the deviation from the herd averages were pooled, a barely statistically significant effect of the J gene was obtained ($P \approx 0{\cdot}05$). However, no cumulative effect of the $B^{BO_1YD'}$ and J gene was found. In SRB the fat percentage of the daughters from the 15 sires carrying both these genes was thus almost exactly the same as in daughters from sires carrying $B^{BO_1YD'}$ only.

With regard to the L system finally, the results were conflicting. Within groups of paternal half-sibs of the SRB breed the L gene seemed to exercise

Table 1. INFLUENCE OF SOME BLOOD GROUP GENES CARRIED BY A.I. BULLS ON THE BUTTER-FAT TEST OF THEIR PROGENY*

Breed	Blood group genes	No. of sires	Fat percentage of daughters	t	Difference between progeny average and contemporary herd average	t	Weighted difference between paternal half-sibs having and lacking respectively genes (ref. 2)
SRB	With $B^{BO_1YD'}$	37	4·23	3·42‡	+ 0·11	3·66‡	+ 0·19‡
	Without $B^{BO_1YD'}$	168	4·12		+ 0·01		
	With J	62	4·17	1·84	+ 0·06	1·90	+ 0·08†
	Without J	143	4·13		+ 0·02		
	With L	27	4·14	0·06	+ 0·03	0·21	− 0·14†
	Without L	178	4·14		+ 0·03		
SLB	With $B^{BO_1YD'}$	5	4·07	2·47*	+ 0·10	2·54*	—
	Without $B^{BO_1YD'}$	70	3·96		+ 0·01		
	With J	14	4·01	0·98	+ 0·04	0·87	—
	Without J	61	3·96		+ 0·01		
	With L	27	4·02	2·28*	+ 0·07	2·80†	—
	Without L	48	3·94		− 0·02		

* $P < 0{\cdot}05$. † $P < 0{\cdot}01$. ‡ $P < 0{\cdot}001$.

a negative influence on fat percentage. However, in the present work the daughters from the SRB sires of group *L* had almost exactly the same fat percentage as the daughters from sires lacking *L*. In SLB, on the contrary, the daughters from sires of group *L* had significantly higher fat percentage than those from sires lacking *L*.

Relationships between fat percentage and blood-group genes may be due to pleiotropy or linkage. If due to relatively close linkage, results such as those obtained in the present work with regard to *L* might be possible. The fact that in three independent materials comprising two different breeds the $B^{BO_1YD'}$ and *J* genes were associated with high butter-fat tests suggests that pleiotropic relationships exist between fat percentage and the corresponding two blood groups. In the case of the $B^{BO_1YD'}$ allele the increasing influence on fat percentage seems to be so strong that selection for this allele might possibly be used to raise the fat percentage of the milk. However, before such selection can be recommended in practice, its influence on other production traits must be known.

A full account of this work will be published elsewhere.

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Hæmoglobins of Fœtal CBA Mice

IN man and many other species, fœtal hæmoglobin differs from the hæmoglobin of the adult, the difference being in the globin moiety and not in the prosthetic group[1]. The hæmoglobin in the blood of the new-born is often a mixture of fœtal and adult types. Adult *CBA* mouse blood can be shown, by starch gel electrophoresis, to contain at least 3 hæmoglobins (Cohen, B., personal communication). No one has, apparently, studied the fœtal hæmoglobins of *CBA* or other strains of mice.

In the present investigation, the blood of new-born and of fœtal mice was collected by decapitating the animals in heparinized saline. The red cells were washed twice in saline and finally packed by centrifuging at approximately 1,000*g* for 10 min. An equal volume of distilled water was added to lyse the cells and the cellular debris spun down. The supernatant provided the hæmoglobin samples to be investigated. Adult hæmoglobin solutions were prepared from the mother's blood in a similar manner.

The resistance of hæmoglobin to denaturation with 0·01 *N* sodium hydroxide has been investigated by Welling and van Bekkum[2], and our results on adult *CBA* hæmoglobin, observing the denaturation of diluted solutions in the spectrophotometer at 576 mμ, are comparable with their published figures. The hæmoglobins from new-born and fœtal mice were not sufficiently different from adult hæmoglobins in their resistance to the alkali to allow them to be readily distinguished; however, there appeared to be only two components in the curve for fœtal hæmoglobin while many of the samples of adult hæmoglobin showed an extra fast component. Similarly, no satisfactory differentiation could be made between these hæmoglobin solutions using the technique of oxidation to methæmoglobin with either 0·01 or 0·02 *M* sodium nitrite or with 0·0005 *M* potassium ferricyanide. The progress of the oxidation of dilute solutions of hæmoglobin was again followed at 576 mμ. Betke[3] has found that this technique could be used to characterize the human fœtal and adult hæmoglobins.

Starch-gel electrophoresis has been successful in identifying different hæmoglobin types of human[4] and mouse[5] blood. Accordingly, samples of hæmoglobins of fœtal, new-born and adult *CBA* mice were examined in this way. Particular care was taken with the early fœtal samples to ensure that no contamination of the fœtal blood with maternal or placental blood occurred. Washed and packed cell samples were lysed in their own volume of distilled water; *M*/1 phosphate buffer, *p*H 6·8, was added to double this volume and the solution centrifuged. The supernatant hæmoglobin solutions were converted to the stable cyanmethæmoglobin[6] form using potassium ferricyanide and potassium cyanide, and were then inserted into the starch gel on filter paper wicks. The Poulik discontinuous buffer system[7] was used with a current of 20–30 m.amp. at 6 V./cm. gel supplied by a low impedance, constant voltage, power pack. The current was passed for 6 hr.; the gel was then sliced and stained with a hydrogen peroxide/*o*-dianisidine solution[8]. Electrophoresis of the hæmoglobins was always carried out during the same day that the animals were killed. As can be seen from Fig. 1, the early fœtal hæmoglobins show a distinctive pattern. The samples of hæmoglobin from late fœtuses and new-born *CBA* mice were not, however, consistently different from those of the mothers. Further work on pregnancies, dated by

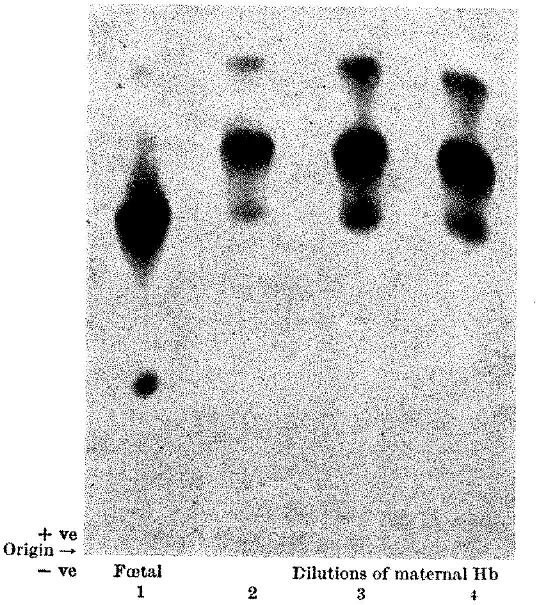

Fig. 1. Starch-gel electrophoresis (Poulik discontinuous buffer system, ref. 7) of cyanmethæmoglobins prepared from early fœtal and from three dilutions of maternal *CBA* mouse blood

vaginal plugs, showed that the early fœtal pattern was seen from the twelfth to the fourteenth or fifteenth day of inter-uterine life, but that from the sixteenth day onward the adult pattern was generally obtained. The early fœtal pattern differs from the adult in a diminution of the relative amounts of the fastest moving band and a predominance of the third rather than the second component. The slowest-moving band was well marked in the early fœtal samples but was also present, though irregularly, in later life: the significance of this slowest band needs to be investigated further.

It can thus be seen that, in *CBA* mice, early fœtal blood may be distinguished from later fœtal, new-born or adult blood by the relative distribution of the different hæmoglobins.

We wish to thank Mr. Bernard Cohen of the University of Glasgow for his help and advice during the course of this investigation.

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PATHOLOGY

Brain Glycogen- and Copper-Levels in Normal Sheep and Sheep affected with Scrapie

Little is known about the biochemical changes responsible for the characteristic symptoms of scrapie. We intend to examine systematically the brain and other tissues for evidence of biochemical derangements. A communication describing the lack of alteration of the serum proteins has already been published[1].

Attempts to relate disease conditions in domestic animals to those in man can be most informative. While scrapie has been likened to kuru, a disease affecting tribesmen in a localized area of New Guinea[2], we have considered, in view of the familial nature of scrapie[3], that scrapie may be related to two familial conditions in man, one a glycogen storage disease (Castaigne-Lhermitte) and the other associated with an excess of copper in certain organs (Wilson's disease)[4].

In Casteigne-Lhermitte's disease, a familial cardio-muscular variant of Von Gierke's hepato-renal glycogen storage disease, histological examination of the nervous system reveals the accumulation of glycogen within the nerve cells, varying from small droplets to confluent masses. The latter may actually displace the nucleus. This raises the question as to whether these could be confused with the neuronal vacuoles characteristic of scrapie. In individuals with the glycogen storage disease there is a motor disability characterized by muscular hypotonia. Death usually follows from bulbar paralysis or respiratory infection. Somewhat similar nervous symptoms may occur in scrapie.

Wilson's disease is a familial disease characterized by progressive rigidity, a coarse tremor of the limbs and a spastic condition of the face, mouth and pharynx with some degree of dementia in the final stages. A few cases develop in the first decade, but the onset is most common in the second decade. The disease is associated with the finding of a great excess of copper in the liver, corpus striatum, and the cornea. This is due to the failure of the serum globulins to bind copper as it is absorbed from the intestine. While there are differences in symptomatology between this disease and scrapie, the importance of copper in sheep neuropathology[5] suggested the necessity for basic information on copper-levels in sheep affected by scrapie and normal sheep.

Six sheep in which symptoms of scrapie had been experimentally induced were killed, the brains frozen within 60 min. and kept frozen until analysed. Nine normal brains from a packing house and similarly treated were used as controls.

For glycogen, 1 gm. of the frozen brain was weighed into alcoholic potassium hydroxide and hydrolysed by Kerr's method[6]. The glycogen was separated by centrifugation, taken up in trichloroacetic acid solution and analysed by the anthrone procedure described by Kahan[7].

For copper, 15 gm. of sheep brain were ashed overnight in platinum at 600° C. The residue was repeatedly digested with nitric acid, dried and returned to the muffle at 600° C. for 1 hr. until white and then made up to volume with dilute hydrochloric acid. The final determination was carried out on a suitable aliquot by Forster's method[8].

Brain glycogen values of the six sheep affected by scrapie ranged from 0·86 to 1·04 mgm./gm. of dry matter while the range for nine normal brains was 0·57–1·18. The early onset of scrapie, differences in symptomatology and the fact that brain tissue from affected sheep does not show elevated glycogen-levels indicate that scrapie is not similar to Castaigne-Lhermitte's disease.

Brain copper values of affected sheep ranged from 12·2 to 16·7 parts per million of dry matter while the range for normal brains was 13·0–35·0. The values for scrapie sheep thus fall within the lower part of the normal range. This indicates that a defect in copper metabolism is unlikely to occur in the pathogenesis of scrapie and that there is little chance of a relationship to Wilson's disease.

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Isolation of *Trypanosoma rhodesiense* from Wild *Glossina palpalis*

In an account of the epidemiology of human trypanosomiasis in south-east Uganda, Robertson and Baker[1] have referred to the momentous discovery by Bruce *et al.*[2] of the association between *Glossina palpalis fuscipes* Newstead and the devastating epidemic due to *Trypanosoma gambiense* in the early years of this century. Before the occurrence of *T. rhodesiense* Stephens and Fantham in epidemic form in 1940 [3] and its persistence afterwards, sleeping sickness in south-east Uganda was due apparently to *T. gambiense* and was considered to be transmitted only by *G. palpalis*.

During the epidemic of *T. rhodesiense* MacKichan[3] recorded the first isolation of this species from *G. pallidipes* Austen in the Lugala area of south-east Uganda and afterwards other isolations of *T. rhodesiense* from *G. pallidipes* have been made[4,5]. The interesting change from *T. gambiense* sleeping sickness to that due to *T. rhodesiense* and the similarity of the epidemiological picture of both forms of the disease in south-east Uganda have been commented on by Robertson and Baker[1].

Unsuccessful attempts to isolate *T. rhodesiense* from *G. palpalis fuscipes* have been recorded[3-5]. Rennison[4] failed to isolate any *T. brucei* group trypanosomes, and of Willett's five strains, the four which were tested proved non-infective to man.

During January and February 1960, 14,500 *G. palpalis fuscipes* were collected at Lugala (0° 12′ N.; 33° 54′ E.) in south-east Uganda, and allowed to feed, not merely to probe, in batches on guinea pigs and albino rats. From these animals 21 strains of the *T. brucei* group were isolated; no other species were detected. These strains were sub-inoculated into albino rats and samples of the blood of the latter animals, killed at high parasitæmia, were frozen and stored in a dry-ice cabinet.

Morphologically all the strains were of the *T. brucei* group and were readily established in albino rats. The mean prepatent period in rats, sub-inoculated with blood from the animals in which the strains were originally isolated, was 3·9 (range 2–12) days. The parasitæmias reached 50 or more trypanosomes per high-power field in a wet preparation (4 mm. objective, × 10 ocular) in a mean of 8·6 days. The mean longevity of these infected rats was 18·0 (range 8–48) days.

The strains were tested for sensitivity to tryparsamide (B.P.) by injecting mice, at high parasitæmia, intraperitoneally with tryparsamide, at the rate of 160 mgm./kgm. on the sixth day after inoculation; the parasitæmias were little affected and none of the animals was cured.

In order to discover whether any of the 21 virulent, tryparsamide-resistant *T. brucei* group strains were in fact *T. rhodesiense* the infectivity to man had to be ascertained. It was not possible to test each strain individually, so strains were pooled and inoculated into one man as described below.

The possibility of improvement of the condition of a patient with parenchymatous neurosyphilis by fever therapy was being considered. As *T. rhodesiense* produces an acute fever and the infection is curable in its early stage with suramin (B.P.), it was considered justifiable to combine a test of these strains with this therapy. The patient had a strongly positive Kahn test and showed serious mental deterioration. He did not come from a sleeping-sickness area.

Table 1. Batches in which 21 Strains of the *Trypanosoma brucei* Group were Inoculated into Man

Batch	Strain No.	Estimated No. of trypanosomes inoculated (millions)	Site of inoculation	Chancre
A	5	8·0	Left thigh	absent
	8	38·0		
	13	27·2		
B	1	0·6	Right thigh	present
	2	63·1		
	4	4·2		
	7	15·6		
	9	14·6		
	10	8·6		
C	11	16·8	Left arm	present
	12	5·5		
	14	10·5		
	15	30·6		
	16	23·1		
	17	4·7		
D	18	13·7	Right arm	present
	19	14·6		
	20	13·3		
	21	14·3		
	22	21·3		
	23	17·6		

The 21 animals, infected each with one individual strain from the frozen material, were grouped into four batches (Table 1). To a vial of 2·5 ml. sterile heparinized Ringer's solution was added 0·1 ml. of heart blood from each member of batch *A* (Table 1). After mixing, 2·0 ml. of this suspension were inoculated subcutaneously into the patient. This procedure was repeated for each batch *B*, *C* and *D*; the inoculations were made successively on day 0 at about 2-hr. intervals at the sites designated in Table 1.

The patient was examined each morning and thick blood films were examined daily. Trypanosomes were detected in the patient's peripheral blood on day 7 before a chancre had been seen. On day 8 characteristic chancres were seen at sites *B*, *C* and *D*. On day 9 the peripheral blood was estimated to contain 420,000 trypanosomes per cu. mm. A slight pyrexia (axillary temperature, 99·2° F.) occurred on the evening of the day 7 and was followed by a continued pyrexia (axillary temperature, 102·4°–104·0° F.) from day 8 to day 9, when the patient was treated with suramin. The temperature subsided by crisis on day 10. The patient's mental condition appeared a little improved.

To summarize these observations; all the strains isolated were highly virulent to rats or mice in the laboratory and were resistant to tryparsamide. In addition, at least one of the strains produced an acute infection with a high parasitæmia in man after a short incubation period. The appearance of chancres at three of the four sites of inoculation suggested that each of batches *B*, *C* and *D* contained at least one strain infective to man.

It is concluded that among the strains there was at least one strain of *T. rhodesiense*, possibly more.

This is the first isolation of *T. rhodesiense* from wild *G. palpalis*; furthermore, it is the first isolation of a trypanosome proving infective to man from wild *G. palpalis*. Taylor's[6] presumptive evidence that the salivary infection in a *G. palpalis* at Ganawuri was *T. gambiense* is discussed by Buxton[7]. The widely held view that *G. palpalis* is only associated with *T. gambiense* sleeping sickness must clearly be re-examined.

We wish to express our appreciation of the encouragement given by Dr. W. H. R. Lumsden, director of the Organization, to epidemiological studies which have led to this result. Acknowledgment is given to Dr. P. E. C. Manson-Bahr and Mr. A. E. C. Harvey for their co-operation and help.

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RADIOBIOLOGY

Quantitative Cytochemistry using Directly Applied Radioactive Reagents

While radioactive isotopes have found valuable application in many cytochemical studies of metabolic incorporation processes, little use has so far been made of such labels in cytochemical reactions applied directly to the localization of substances in tissue specimens. Some qualitative applications to enzymic cytochemistry, for example, using metals or phosphorus-32 in deposited products, have been reported[1]. We have now examined the applicability of tritium-labelled reagents which form covalent bonds at specific sites in tissue components, as a method in quantitative cytochemistry. The advantages of such a method will include, *inter alia* (cf. ref. 2): (1) an escape from some of the problems of selectively introducing sufficient light-absorption at specific sites, since the label can be carried on many suitable simple reagent molecules; (2) autoradiographic measurement, which is free of certain of the problems of micro-spectrophotometry and is independent of the molecular environment of the target sites; (3) checks and calibrations of micro-spectrophotometric methods for the same components, and the possibility, at least in principle, of absolute determinations.

To establish the basis of the method, the acylating reagent acetic anhydride, labelled with tritium, was applied (0·4 *M*) in pyridine-acetonitrile as a general blocking agent[3], to indicate the total number of groups available to acylation, notably $—NH_2$, $—OH$ and $—SH$ groups in macro-molecules. Frog red cells (in plasma-free smears) were exposed to a layer of reagent solution in small, closed tubes. Thorough subsequent washings, in turn in unlabelled reagent, sodium acetate solution and water, removed all excess isotope. Satisfactory and reproducible autoradiographs were obtained, by the stripping film method of Pelc[4], after short film exposures (for example, 2 days, for reagent at 1·5 $\mu c./\mu M$). The amount of isotope bound per cell increased with the reaction period, up to a maximum value (Fig. 1). Nil counts above background were found in cells similarly treated but having a prior treatment (15 hr.) with unlabelled reagent.

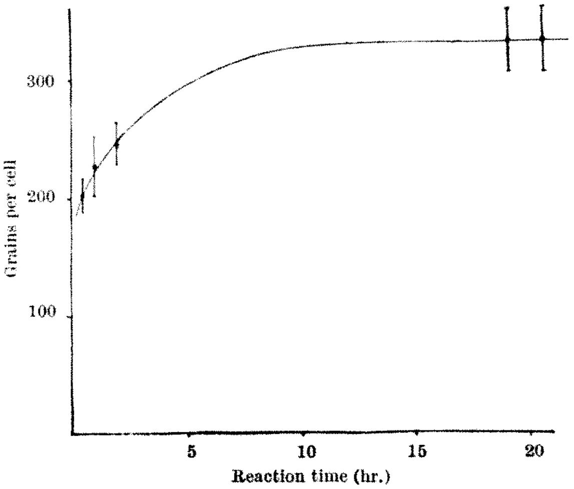

Fig. 1. Mean grain count (± *S.D.*) per cell, in normal frog red cells, after different periods of reaction

The total number of groups (N) available to this reagent in the normal frog red cell (lengths of axes in the ranges 20–23μ and 13–15μ) was found to be $5 \cdot 8 \times 10^{-13}$ mole (or $3 \cdot 5 \times 10^{11}$ groups). This is consistent with reaction at a large majority of the potential acceptor sites in the macromolecules in this cell. Such a figure is calculated from the equation:

$$N \text{ (moles per cell)} = \frac{2\, n_{\max.}}{g_1.g_2.a.t.s.},$$

where $n_{\max.}$ is the maximum number of grains that can be developed per cell after treatment with reagent at specific activity s (disintegrations per min. per mole) and film exposure of t min.

g_1 and g_2 are grain-yield correction factors. g_1 is equal to the mean number of grains actually developed per activating β-particle. Multiple grain activations are betrayed by close clusters of 2 or 3 grains, and an idea of the magnitude of g_1 is obtained by scoring these as units. Such re-counting gave $g_1 = 1 \cdot 28$ (*S.E.* 0·03) which, in view of the inclusion of accidental clusters, is the upper limit. g_2 corrects for all those incident β-particles not producing grains, due to latent image fading, low energies, multiple collisions, etc. In practice, only $g_1.g_2$ is required, and this product was determined empirically by comparison of the emergent β-particle flux from a specimen (read in a gas-flow counter) with the number of grains developed in an autoradiograph of the same specimen. This ratio gave a value of 0·85 for $g_1.g_2$.

Self-absorption is represented by the absorption correction factor a. Assuming the absorption data of Fitzgerald *et al.*[5] hold here, and if the equivalent mass per unit area of the cell is known (found here by interferometry[6]), the total absorption can be obtained by an appropriate integration. This method gives $a = 0 \cdot 21$ here (that is, 21 per cent transmission). A direct determination of a was also possible, by measurement of the emergent β-particle flux from a specimen, followed by extraction and measurement (in a gas-flow counter) of the total radioactivity of that specimen. Two such experiments gave values of 0·18 and 0·19 for a. The directly determined value was preferred in calculations of N.

For comparative work, the correction factors evaluated here can be ignored, but for absolute determinations they must be investigated for each new type of material. The routine use of standard labelled specimens, for example of acylated frog red cells as here, is useful as a means of monitoring variations in the autoradiography of other treated specimens.

Similar methods can clearly be applied with other reagents for the determination of important types of groups in various cells. We have found that the number of available protein $-NH_2$ groups can apparently be measured by use of the same reagent in cold, aqueous buffer (cf. ref. 7). With the use of unlabelled, selective blocking agents, or with the introduction of labelled acetyl into other reagents, or with the use of reagents of other types labelled with tritium (or carbon-14 or sulphur-35 where the highest resolving power is not required), the measurement of other cell components will be feasible.

A fuller account and discussion of these and related experiments will appear elsewhere. We thank Dr. S. R. Pelc for discussion, and the Medical Research Council for a grant to support this work.

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Modification of Radiation Damage in Seeds by Post Treatment

IN their recent paper on modification of radiation damage in dormant seeds by storage, Curtis *et al.*[1] reported that very dry barley seeds if germinated immediately after irradiation show the same damage as seeds of higher water content. The very dry seeds, however, as distinct from those of higher water content, showed a considerable storage effect, the damage increasing as the seeds are stored between irradiation and soaking. In these experiments soaking was carried out under anaerobic conditions for an initial period of 1 hr.

The existence or absence of a storage effect in irradiated seeds has been reported by many workers[2]. However, the moisture content of the seeds, which greatly influences their radiation sensitivity[3], was not controlled in most of these earlier investigations. In the remaining papers there appears to be no agreement on how the observed storage effect is related to the water content or how it is influenced by the conditions of storage and soaking. In order to shed some light on these questions, a study of the storage effect in *Vicia faba* seeds was therefore undertaken using precisely controlled experimental conditions.

The material used was *Vicia faba* var. *minor* 'Throws M.S. Winter Beans', kindly supplied from Hasler and Co., Ltd., Dunmow, Essex. The beans were stored in desiccators in single layers over sodium hydrogen sulphate or calcium chloride, the water content being measured by crushing samples of 20 and drying at 105° C. to constant weight.

Seeds of different water content were pricked and given 3,000 r. or 10,000 r. X-rays, using 200 kV., 15 m.amp., 2 mm. aluminium, 550 r./min. Irradiated seeds and controls were either soaked 4 min. after the end of irradiation for an initial 8-hr. period, or stored for 8 weeks in desiccators and then soaked. Water content was kept constant during storage. It can be shown that, after 8 hr. soaking, 90 per cent of embryos are fully soaked, whole seeds having taken up about 85 per cent of their original dry weight of water. The soaking was done in quartz-distilled water at 20 ± 1/4° C. Either air free of carbon dioxide, or nitrogen free of carbon dioxide and oxygen (purified product of Linde A.G. with oxygen content of less than 0·001 per cent, purified further by passing over catalytic copper[1]) was bubbled through the water during soaking.

Seeds were then grown in pots of moist sawdust in an incubator running at 20 ± 1/4° C. and 90–95 per cent relative air humidity. Germination was determined and root and shoot length measured 11 days after soaking. The beans were stored in darkness except for the final pricking and irradiation, and soaked and grown in darkness except for 10 min. daily at less than 20 ft.-candles. These short exposures to light should not affect the ultimate length of the shoot[5,6]. For each experimental procedure three pots of 20 beans were used. The significance of the resulting differences was tested by means of analysis of variance.

For seeds of 9·7 and 10·4 per cent water content the different conditions of soaking after irradiation had no effect on the percentage germination using either of two criteria: (*a*) visible splitting of the testa over the tip of the radicle; (*b*) root- or shoot-length longer than 0·5 cm.

Root and shoot growth results for the same experiments are summed up in Fig. 1. The root and shoot growth is expressed as a percentage of the individual controls in Table 1.

Table 1. Length of root and shoot after 11 days growth expressed as a percentage of individual controls for beans of 9·7 per cent moisture content, irradiated with either 10,000 r. or 3,000 r. and soaked immediately or after 8 weeks storage, in either nitrogenated or aerated water

Soaking time	Soaking procedure	3,000 r.		10,000 r.	
		Root	Shoot	Root	Shoot
4 min. after end of irradiation	Nitrogen	63·9	45·3	21·5	7·6
	Air	66·2	43·9	22·1	8·3
8 weeks after end of irradiation	Nitrogen	58·0	38·3	26·1	10·3
	Air	64·8	41·2	23·5	8·4

It can be seen that there was a strong effect of both irradiations on root- and shoot-length, but no clear effect of the other factors in question. Statistical analysis shows that apart from the irradiation ($P \ll 0·001$) only moisture content gave significant differences in root and shoot length ($P = 0·01$–$0·001$). Seeds with 9·7 per cent humidity at zero soaking time gave slightly shorter roots and shoots than seeds with 10·4 per cent humidity. This damaging effect of drying in *Vicia faba* var. *minor* seeds was pointed out earlier[7–9]. According to the new results it appears to be significant for very small differences in water content. It should be mentioned, however, that growth reduction in the hybrid stock used here at 9·7 per cent water content was small, com-

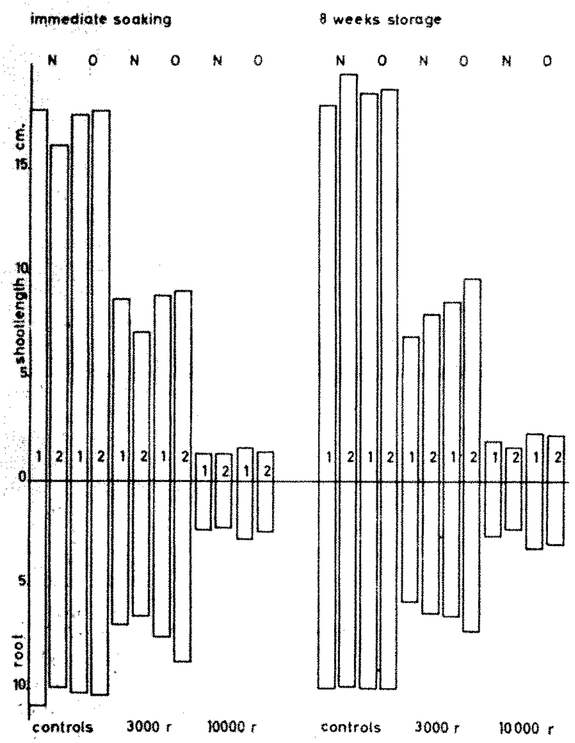

Fig. 1. Shoot- and root-length of seedlings eleven days after soaking. Seeds of two moisture contents, 9·7 per cent (1) and 10·4 per cent (2). Doses of 3,000 r. and 10,000 r. X-rays. Soaking, 4 min. or 8 weeks after end of irradiation, under aerobic (*O*) or anaerobic (*N*) conditions. Means from 60 beans in each case

pared with the earlier findings for an inbred line and that seeds of 15·1 per cent water content gave no better growth than those of 10·4 per cent.

Apart from these two effects there was no other significant effect. Neither the period of storage, nor aerobic or anaerobic soaking had any effect on the radiation damage observed, nor was any higher interaction observed between radiation and storage, or radiation, storage and soaking procedure. It should be mentioned that *Vicia* seeds of higher moisture content (12 per cent) when irradiated with 3,000 r. gave no growth reduction (experiments carried out in Southampton, unpublished), which shows that the water content used here (9·7 per cent, giving about 42 per cent growth reduction in shoots for 3,000 r.) was of the right order for *Vicia* seeds for testing the effects in question.

The above results, though at variance with those of Curtis *et al.*[1] and other workers on barley in showing no post-radiation effect of storage or of availability of oxygen during soaking, do not preclude the possibility that these effects may occur in *Vicia* under different conditions. Curtis *et al.* used barley seeds dried to very low water content; they gave higher doses and grew their seedlings in the light. However, it seems important to point out that any generalization concerning 'storage effect' and 'modification by post-treatment' is at present premature, and it is clear that further experiments of a wider scope and with other materials are necessary in order to determine the conditions under which such effects appear.

I am indebted to the Department of Scientific and Industrial Research and the Deutsche Akademische Austauschdient for a research fellowship in Great Britain, during which earlier stages of the work reported here were carried out. Sincere thanks are due to Dr. G. R. Lane of the Botany Department, University of Southampton, for valuable suggestions and constant encouragement during the period of study there, as well as for very carefully going into part of the experimental data and for help in the English manuscript. I am also indebted to Dr. L. H. Gray, Northwood, Middlesex, and to Dr. H. J. Seifert of the Chemistry Department, Giessen, for kind suggestions regarding the gases used. Irradiations were done at Southampton by Dr. J. R. Clarkson of the Royal South Hants and Southampton Hospital, at Giessen by Prof. Th. Hornykiewitsch and Dr. W. Hartung of the Medizinische Klinik der Universität. Their kind co-operation is gratefully acknowledged. The work in Giessen is supported by the Deutsche Forschungsgemeinschaft. A more detailed account will be given elsewhere.

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BIOLOGY

Rapid Preparation of Fresh Frozen Sections of Barley Roots

In the course of investigations of the movement of nutrient ions through barley roots, it became necessary to stop the movement instantaneously and study the distribution of the nutrient ions at that time. A technique was devised that involved stopping the reaction at liquid-nitrogen temperature and sectioning the frozen tissue for subsequent radioautography. The purpose of this communication is to report on a method of preparation of fresh frozen sections involving no prior fixation of the tissue.

The barley roots are placed in water at 0–5° C. for 1 min. They are then oriented in a slightly congealed 5 per cent gelatin mixture containing 3·5 per cent glycerine jelly. The matrix is prepared by mixing 50 c.c. of melted 10 per cent gelatin mounting medium containing 0·5 per cent carbolic acid ('Harelco'), 7 c.c. of glycerine jelly mounting medium (Brandt), and 50 c.c. of water. The mixture is allowed to cool to a soft jelly and spooned into a small embedding box that has been chilled in 'dry ice'. The roots are immediately pushed into the 'jelly', oriented and the whole embedding box placed in *iso*pentane that has been cooled by liquid nitrogen to approximately −160° C. If the drop in temperature is too rapid, the matrix blocks will often crack, and at least a minute should be allowed to bring the blocks to −160° C.

After freezing, the blocks are left at room temperature for a few minutes to allow them to be cut from the embedding boxes and then placed in a cryostat kept at approximately −22° C. When tissue blocks are brought up to this temperature, recrystallization begins to occur, although rather slowly[1]. Nevertheless, within two days the cutting characteristics of the tissue blocks change noticeably. Very low-temperature storage is advisable if blocks are not cut within a day or two. The blocks are mounted and cut at −20° to −22° C. The Minot microtone, lubricated with 'Arctic' oil, has been satisfactory, and 16-μ sections of barley roots can be cut as a matter of routine. The use of a glass window[2,3] on the knife prevents cutting the section.

Figs. 1 and 2 show cross-sections and transverse sections of water-mounts cut according to the above procedure. The photographs were taken within fifteen minutes of the tissue being cut. There is no indication of physical alteration of the tissue during preparation or cutting. The cross-section and transverse sections are similar to the illustrations of Heimsch[4] of barley roots prepared by standard techniques involving paraffin impregnation.

After sectioning, alternate procedures are possible depending on the use to be made of the section. The section may be brushed on to a warm gelatinized slide, where melting and flattening take place at once. The sections dry very rapidly, and solutions such as water or vital dyes should be added immediately. A procedure found successful for morphological detail is to brush the section on to a warm gelatinized slide flooded with 10 per cent neutral formalin and allow it to dry at room temperature. Sections may then be stained and mounted by standard procedures. For radioautography sections may be brushed directly on to photographic plates.

One of the advantages of the present procedure is its rapidity. A sample of fresh plant tissue can be excised, frozen, cut and a section obtained on a microscope slide within an hour, depending primarily on the length of time necessary to raise the blocks from −160° to −22° C.

Another advantage is that a chemical, and to some extent physical, reaction can be stopped or slowed at any given time and the tissue examined within the hour. In our own case, we are interested in ion movement in tissue. We can stop this reaction, and by the use of radioautographs localize the presence of the ions. Other applications in chemical studies, enzyme analyses, and micro incineration are as applicable to plant tissues as to animal tissue prepared by similar techniques[5].

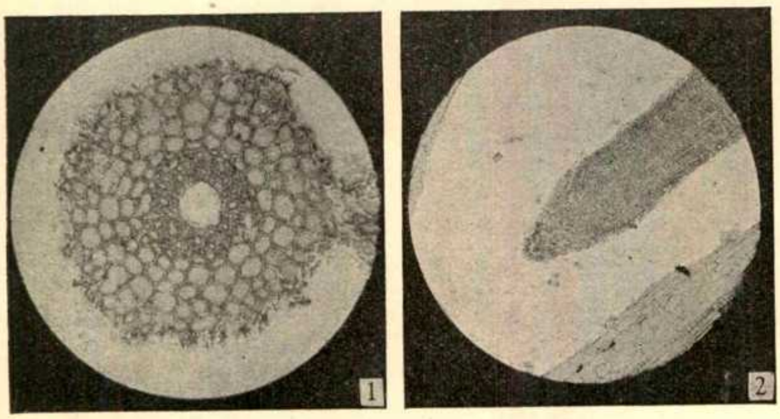

Fig. 1. Water mounts of 16-μ sections of six-day-old barley roots obtained by cutting at −22° C. a frozen gelatin block containing the roots: (1) cross-section, approximately × 400; (2) transverse sections, approximately × 150. Root tip in centre

The present method prepares fresh frozen plant-sections involving no prior fixation or dehydration of the tissue, in contrast to other frozen tissue techniques requiring drying[6] or prior fixation. Thus, artefacts and changes occurring during tissue preparation are minimized to vanishing point.

Although the method has been used only on roots of young barley plants, it shows promise as a general method for the rapid preparation of fresh tissue sections without prior fixation.

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Low-Temperature Injury in Lovegrass (*Eragrostis curvula* (Schrad) Nees)

Eragrostis curvula is a perennial bunch grass widely used in South Africa. In order to determine the low-temperature tolerance of this grass, the following experiment was carried out. Twenty dormant stools of *E. curvula* ('Ermelo' variety), cut to within 3 in. of the ground, were transplanted in the winter (May 28) of 1958 into 4-gal. tins, and watered. Ten of these tins were placed in an illuminated constant-temperature room at 31° C. (87·8° F.) on July 9. Active growth started within a day, and the plants reached a height of more than 12 in. within nineteen days. On July 28 the plants were grouped in pairs, each of which was subjected to one of the following temperatures for three days: − 16·7° C. (2° F.), − 13·3° C. (8° F.), − 12·2° C. (10° F.), − 10·8° C. (12·5° F.) and 1·7° C. (35° F.). They were then transferred to a glasshouse (at approximately 24° C. (75° F.)) for further observation. All stools had been killed by low temperature.

Of the remaining ten tins, two were placed in each of the illuminated low-temperature rooms maintained at temperatures of −16·7° C. (2° F.), −13·3° C. (8° F.), −12·2° C. (10° F.), −10·8° C. (12·5° F.) and 1·7° C. (35° F.) on July 9. On July 28 these tins were transferred to high-constant-temperature rooms (31° C. (87·8° F.)) for three days, after which they were placed in a glasshouse (approximately 24° C. (75° F.)) for further observation. Only the two plants which had previously been maintained at 1·7° C. (35° F.) resumed growth within two days, the remainder having been killed.

These observations support the views of Crider[1], who suggested that most perennial crop plants were subject to greater winter injury if previously encouraged by irrigation or other means to remain in a succulent condition until cold weather set in, and further that winter injury could not be attributed to low temperature alone.

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Effect of Polyhydric Alcohols on Frost Hardiness in Plants

In previous communications[1-3], I have reported that the increase of sugar content in a plant cell increases its frost hardiness, and that polyhydric alcohols, especially ethylene glycol and glycerol, also show the same protective action as glucose and sucrose against freezing injury in *Gardenia* leaf cells.

Recently, some attempts[4,5] have been made to determine the correlation between the seasonal fluctuations of glycerol content and frost hardiness in some insects. However, no evidence has yet been obtained to show whether the increase in polyhydric alcohols, especially in glycerol, is an important cause of frost hardening in plants or not. Therefore, the fluctuations of various polyhydric alcohols in the bark of twigs or leaves of 19 different species of woody plants were studied chromatographically in the course of both natural and artificial frost hardening. An aliquot of the extract of high concentration of polyhydric alcohols, prepared by the usual method, was developed on a filter paper, using a solvent containing *n*-butanol–water–acetic acid (4 : 1 : 2). In order to separate mannitol and sorbitol, the process was repeated five times. Quantitative analysis of three polyhydric alcohols was carried out, based on oxidation with periodate of the polyhydric alcohols eluted from the filter paper and subsequent colour reaction with a chromotropic reagent.

In winter, the glycerol content in tea, climbing spindle tree and *Platanus* ranged from 0·1 to 0·15 per cent (wet weight), while in the other 16 species its content was very small (Table 1). Furthermore, glycerol content showed only a slight change which was obviously not significant, in both seasonal and artificial hardening. Therefore, it may be safely said that glycerol does not play an important part in the frost hardening of woody plants.

In winter, the content of mannitol ranged from about 0·1 to 0·5 per cent (wet weight) in many species (Table 1). Sorbitol content amounted to about 1 per cent in the apple tree (twig bark), *Gardenia* (leaf), pomegranate (twig bark) and mountain ash (twig bark), but its content is very small in the other 15 species (Table 1). In winter the contents of mannitol and sorbitol in many species increased 2–3 times as much as in August; but in the mulberry tree they were reduced to a half the summer value. From summer to winter, the increase of total polyhydric alcohols in bark of twig of apple tree was about 38 per cent of that of total sugars during that period, but the values of all other 6 species used were less than about 7 per cent.

The total content of polyhydric alcohols in the pomegranate (bark), *Gardenia* (leaf), apple tree (bark) and mountain ash (bark) in winter ranged from about 40 to 55 per cent of the total sugar content, but in the other 15 species it does not amount to more than 12 per cent (Table 1).

In artificial hardening in early October, when such hardening is remarkably effective, the increase in polyhydric alcohols did not exceed about 0·1 per cent (wet weight) in many species; at the same time, increase in sugars ranged from about 1·0 to 1·6 per cent (wet weight) in all species. Moreover, any positive correlation between the amount of polyhydric alcohols and the hardiness of trees was not observed in 17 species examined (Table 1).

From the results mentioned above, it is obvious that in both natural and artificial frost hardening polyhydric alcohols play little part in the increase of the frost hardiness in most woody plants. However, in the species in which the total content of polyhydric alcohols amounts to about 40 per cent of the total sugar content, namely, in the *Gardenia*, apple tree, mountain ash and pomegranate, polyhydric alcohols

Table 1. Content of Various Polyhydric Alcohols and Sugars in Twig Bark or Leaf of Woody Plants in Winter

Species	Mannitol	Sorbitol	Glycerol	Total polyhydric alcohol (P)	Total sugar (S)	$\frac{P}{S} \times 100$	Frost hardiness (freezing for 24 hr.)	Material used
Betula tauschii Koidz.	0·23	0·05	0·07	0·35	5·23	6·74	−30° C.*	twig bark
Salix koriyanagi Kimura	0·20	0·06	0·08	0·34	5·40	6·34	−30° C.*	twig bark
Populus nigra L. var. *italia* Muenchh.	0·14	0·05	0·02	0·21	4·69	4·44	−30° C.*	twig bark
Larix leptolepis Murray	0·40	0·09	0·03	0·51	5·29	9·70	−30° C.*	twig bark
Taxus cuspidata Sieb. et Zucc.	0·16	0·10	0·06	0·31	4·27	7·15	−30° C.*	leaf
Pinus pentaphylla Mayr.	0·13	0·03	0·06	0·22	3·97	5·60	−28° C.	leaf
Rosa pendulina L.	0·35	0·06	0·04	0·45	5·26	8·55	−28° C.	twig bark
Rhododendron fauriae Franch. var. *roseum* Nakai	0·18	0·10	0·05	0·22	4·65	4·77	−28° C.	leaf
Abies mayriana Miyabe et Kudo	0·11	0·04	0·08	0·24	4·00	5·89	−25° C.	leaf
Morus bombycic Koidz.	0·07	0·03	0·04	0·15	4·29	3·43	−25° C.	twig bark
Malus pumila Mill.	0·47	1·15	0·03	1·64	3·15	52·10	−25° C.	twig bark
Platanus orientalis L.	0·33	0·08	0·13	0·54	4·77	8·88	−25° C.	twig bark
Euonymus radicans Sieb.	0·14	0·20	0·15	0·45	3·82	11·85	−25° C.	leaf
Punica granatum L.	0·50	0·91	0·05	1·49	3·56	41·80	−15° C.	twig bark
Laurus nobilis L.	0·25	0·45	0·03	0·73	3·61	20·22	−15° C.	leaf
Thea sinensis L.	0·08	0·05	0·12	0·13	3·33	4·00	−15° C.	leaf
Gardenia jasminoides Ellis	0·80	0·59	0·02	1·36	2·54	55·00	−12° C.	leaf
Picea glehnii Mast.	0·07	0·06	0·04	0·16	2·89	5·66	—	leaf
Sorbus commixta Hedlund.	0·35	1·05	0·02	1·42	3·48	40·90	—	twig bark

Quantity of polyhydric alcohols and sugars are expressed in percentage fresh weight. Degree of frost hardiness is represented by the minimum temperature at which the twig or leaves are able to survive freezing for 24 hr. *(At least.) All samples were taken in January exclusive of *Sorbus commixta* and *Picea glehnii*, which were taken on March 1.

are considered to play some part in the increase of their frost hardiness.

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Growth of Lake Victoria Phytoplankton in Enriched Cultures

It has been stated that many of the natural waters of Africa are short of sulphates[1], and as a broad generalization this is not to be denied. However, it has also been suggested that sulphates, as well as nitrates and phosphates, are a limiting factor for plankton growth in Lake Victoria[2], and that shortage of sulphates in solution is a factor affecting the productivity of the Lake[3]. During a recent six-months stay at the East African Fisheries Research Organization Laboratory in Jinja, Uganda, a small part of my time was devoted to culture experiments, and some successful attempts were made to grow Lake Victoria phytoplankton in laboratory culture.

The phytoplankton most usually dominant in Lake Victoria is *Melosira nyassensis* var. *victoriae*, though other diatoms, including *M. granulata*, *M. italica*, *M. agassizii* and *Nitzschia acicularis*, and blue-green algae, including *Aphanocapsa elachista*, *Merismopaedia elegans* and *Lyngbya circumcreta*, are often present. *Melosira* is the most important genus and forms the major source of food, in the Lake itself, for the herbivorous fish, *Tilapia esculenta* and *T. variabilis*, and most attention was paid to *Melosira* in this investigation.

To each of thirteen clean, sterilized, plugged 250-ml. conical flasks was added a 125-ml. aliquot from a fresh, well-mixed, water sample from the Lake. One of these was treated as a control, and to each of the others was added sterile nutrient solution as follows: to cultures S_1, S_2 and S_3, 10 ml. of magnesium sulphate solutions of such concentrations as to produce culture concentrations of, respectively, 1·3, 13 and 130 mgm./l. of sulphate; to cultures P_1, P_2 and P_3, 10 ml. of dipotassium hydrogen phosphate solutions to produce, respectively, 0·874, 8·74 and 87·4 mgm./l. of phosphate; to cultures N_1, N_2 and N_3, 10 ml. of potassium nitrate solutions to produce, respectively, 1·07, 10·7 and 107 mgm./l. of nitrate. To each of a fourth series of three cultures, SPN_1, SPN_2 and SPN_3, was added a mixture of the three nutrient substances at the concentration-levels as described. In calculating these concentrations it was assumed that sulphate, nitrate and phosphate were nil in the Lake water.

Counts of algae were made, by the sedimentation method[4-6] in the original Lake sample, and for each of the thirteen cultures at intervals of six or seven days for the next three weeks. A final count was made for some of the cultures after a further four weeks.

The response of *Melosira* (mainly *M. nyassensis* var. *victoriae*) to some of these different cultures is shown in Fig. 1. No growth occurred in S_1 and S_2, and the difference between S_3 and the initial inoculation density is insignificant. It can be stated, therefore, that added sulphate had no effect on the growth of *Melosira*. Nitrate at 1·07 and 107 mgm./l. had no effect on the growth of *Melosira*, and the cultures N_1 and N_3 are not recorded in Fig. 1. Nitrate at 10·7 mgm./l. (culture N_2) apparently boosted growth initially but after a further six days many dead cells were found in the N_2 culture. On the other hand, the addition of phosphate, particularly in the most dilute form, resulted in a significant increase in *Melosira*. After a further four weeks there were more than 3,000 cells per ml. in culture P_1. *Melosira* also showed a significant increase in culture SPN_3, where all three nutrients were added at the most concentrated level. No growth occurred in SPN_1 or SPN_2; but the reason for this is unknown.

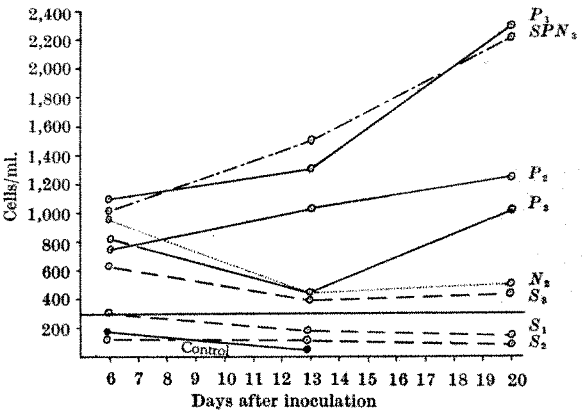

Fig. 1. Effects of added nutrients on growth of *Melosira* spp. in mixed cultures. Horizontal line, original inoculation density

None of the three species of blue-green algae investigated showed a significant increase on the addition of phosphate, nitrate or sulphate; but factors other than nutrient limitations might have been responsible for this.

The Chlorococcales present in the original inoculum, especially *Ankistrodesmus falcatus*, showed an increase in all the cultures with additional nutrients, least so for phosphate and most for nitrate at 107 mgm./l. For these forms, then, the level of sulphate concentration in Lake Victoria might be, with nitrate and phosphate, a limiting factor, and this agrees with the results of culture experiments in which test algae were used[2].

The most important genus, *Melosira*, in the Lake Victoria phytoplankton, however, is apparently not limited by the level of sulphate but is sensitive to phosphate, and hence the biological productivity of Lake Victoria, at the present time, is probably more dependent on phosphate than on sulphate.

I should like to express my gratitude to the Colonial Office for the award of a six-months research fellowship during the tenure of which this work was carried out.

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A Simple Medium for the Demonstration of Chlamydospores of *Candida albicans*

WHILE investigating the formation of chlamydospores by different species of the fungus *Candida*, it was observed that human serum as a medium gave good results. Cultures of *Candida albicans* 10, *Candida tropicales* 10, *Candida krussei* 10, and *Candida stellatoidea* 10, *Candida guillarmondi* 1, and *Candida pseudotropicales* 1, were examined by the following technique: 24-hr. growth in Sabouraud's dextrose agar medium was emulsified with 1 c.c. of sterile normal saline; then 1 c.c. of this emulsion was added to a test-tube containing 4 drops of normal human serum, and the tube was incubated at 37° C. in a water-bath. After a lapse of 24, 48 and 72 hr., cover-slip preparations were examined under the microscope. In each instance, where cultural and biochemical tests for a strain conformed to those for *Candida albicans*, chlamydospores were produced after a lapse of 24 hr. Chlamydospores, however, did not develop with other types. Thus the above findings show that this method is very simple, less expensive and less time-consuming than routine methods.

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Growth of a Nematode in Tomato Plants grown on Sodium-deficient Water Cultures

HOST plants deficient in nitrogen, phosphorus, potassium, magnesium, sulphur and iron will support a population of the root-knot nematode *Meloidogyne javanica*[1], whereas plants on full nutrient and heavily infected with *M. incognita acrita* do not show deficiencies of these elements[2]. It has been reported[3], however, that tomatoes infected with *M. incognita acrita* show less sodium in their tissues than do uninfected plants.

It may be that members of the genus *Meloidogyne* require large amounts of sodium during their development, and the following experiment was undertaken to test the effect of 'sodium deficiency' on the growth and reproduction of *M. javanica* grown in tomatoes. The terms 'sodium deficient' and 'sodium free' used here refer to plants grown in water cultures in which the presence of sodium has been reduced to a minimum.

All seeds used came from a single susceptible tomato plant of the variety Pan America. They were washed six times in water distilled from a silica still which was changed hourly. This water, which was used throughout the experiment, contained 0·0002 p.p.m. sodium.

Nutrient salts were re-crystallized as many as six times, and their sodium content estimated before use. The seeds were germinated on nylon mesh over distilled water. When the radicles were about 1 cm. in length, a culture solution of half concentration containing 0·0042 p.p.m. sodium was substituted for the distilled water. The concentrations of salts used and the methods of growth in polythene containers have been described[4].

Sodium was estimated, using a 'Si-Ro-Spec' atomic absorption instrument[5], on a dry-weight basis. The methods used in infecting the plants and in measuring the growth of the nematodes have been described[1], except that the agar was dialysed against distilled water as a 5 per cent gel for five days with six changes before it was used. A 0·5 per cent solution of the dialysed agar was used as the infecting medium.

Infective larvæ of *M. javanica* were washed several times over a sintered glass filter of pore diameter 5–15μ before being placed in the agar. The plants were exposed to infection for a period of 48 hr. and were then removed. The roots were washed in distilled water before being returned to the nutrients.

The plants were grown in a cooled glass-house from which dust contamination was reduced by a positive pressure of filtered air. A diurnal temperature variation between 60° and 110° F. was maintained during the experiment.

Plants grown in the presence of sodium were grown in culture solutions identical with those without sodium except that they had 0·1 m.equiv./litre of sodium sulphate added. The roots and leaves of these plants were hydrolysed separately in silica Kjeldahl tubes using a mixture consisting of 5 ml. concentrated nitric acid, 1 ml. concentrated sulphuric acid and 1 ml. concentrated perchloric acid, all these chemicals being A.R. grade. Blanks consisting of these chemicals were included in all hydrolyses and sodium estimations.

Throughout the experiment the growth of plants in all treatments was similar. Plants were harvested at 7, 14, 21 and 28 days, and their dry weights were similar, whether or not they were infected or whether or not they had sodium. At the final harvest, average weights of twelve plants from each of the two treatments were approximately the same (6·4 gm.).

The amounts of sodium in the roots and leaves of these plants showed marked differences. Tomato plants grown on a 'sodium free' culture solution showed uniform amounts of sodium which ranged from 20 to 50 p.p.m. throughout the entire experiment for both roots and leaves; the roots always contained sodium in a slightly higher concentration than the leaves.

Plants grown with sodium showed a steady increase in uptake of this element in the roots throughout the entire experiment, ranging from 120 p.p.m. at the first (seventh day) harvest to 1,410 p.p.m. at the final (twenty-eighth day) harvest. The leaves of these plants, however, did not show an increase in sodium concentration throughout the experiment.

These results suggest that sodium accumulates in the roots of tomato plants when the amounts mentioned above of sodium are supplied. Infection was not consistent using agar, and although great care was taken to make this procedure as uniform as possible, in several instances plants remained uninfected.

Infected 'sodium-free' plants were harvested at 7, 14 and 28 days, and galled roots were cut away from the plant and fixed in buffered 1 per cent osmium tetroxide at *p*H 7·2. They were washed in several changes of distilled water and at each harvest fifty nematodes were dissected at random and their cross-sectional area measured as previously described[1].

Growth of these nematodes on tomatoes containing 20–50 p.p.m. sodium was quite normal. They moulted, produced egg sacs, eggs and viable larvæ which were capable of re-infecting tomato seedlings, and it appears that the amount of sodium removed from 'sodium-deficient' tomato plants by light infections (several hundred per plant) of these parasites is negligible, as at the final harvest infected 'sodium-free' plants gave a reading of 40 p.p.m. sodium compared with 35 p.p.m. sodium for uninfected 'sodium-free' plants. From these observations it is clear that *Meloidogyne javanica* does not require a large amount of sodium during its development.

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Mechanism of Trail-laying in Two Species of Termites

DURING recent investigations on the behaviour of the primitive termite *Zootermopsis nevadensis* (Hagen), evidence for trail-laying was obtained when it was noted that nymphs accurately followed the path taken by one nymph over a clear unobstructed surface in an artificial nest, even when the direction of the light and the position of the nest were altered. This, to my knowledge, is the first mention of trail-laying in a lower termite.

Subsequent experiments using squashes and petroleum ether extracts of various parts of the body and organs of individuals of *Z. nevadensis* and *Nasutitermes cornigera* Motschulsky have shown that in these two genera, belonging to two widely separated families (Hodotermitidae and Termitidae), trails are produced by a substance secreted by a gland in the ventral part of the fifth segment of the abdomen. Grassé and Noirot[1], however, from work on *Macrotermes bellicosus* (Smeathman), *Macrotermes natalensis* (Haviland) and *Odontotermes magdalenae* (Grassé and Noirot) believe that in these species trails are marked by fæcal droppings or pellets of earth. In *Z. nevadensis* and *N. cornigera* no trails made with squashes or petroleum ether extracts of the gut, contents of alimentary canal or rectum and drawn on a white card were followed, whereas similar trails drawn with extracts and squashes of the gland produced clear-cut trail-following. *Nasutitermes* does appear to follow a visible trail of fæcal droppings, however, but this is thought to be a consequent phenomenon and not the initial trail marker. When a scarcely visible, by-pass trail, made from the cleaned integument containing the gland, was drawn in competition with a natural trail that had reached the stage where it was quite visibly marked by fæcal droppings, both the workers and soldiers of *Nasutitermes* followed the by-pass in preference to the old trail.

The secretion from the gland has been shown to act as a trail releaser as well as marking the trail.

The trail-laying gland in *Zootermopsis* and *Nasutitermes* appears homologous to that described very briefly in *Kalotermes flavicollis* (Fabr.) by Grassi and Sandias[2], and by Montalenti[3], and in *Rhinotermes nasutus* (Perty) by Holmgren[4]. Grassé[5] has stated that the function of the gland in these species is unknown. In *Zootermopsis* the gland can empty through numerous small pores in the cuticle into a crude external reservoir formed by the almost complete overlapping of the fifth sternite by the preceding one. The nymphs of *Zootermopsis* are often seen dragging their abdomens along the ground when moving, thus bringing segment 5 in contact with the substrate, while the segments at the extremity of the abdomen are clear of the ground. The substance from the gland can thus quite easily escape from the reservoir on to the surface on which the insect is crawling.

All the observations and experiments on *Zootermopsis* were made on laboratory colonies which had been conditioned to live in constant illumination provided by a 'Daylight' fluorescent lamp.

A full account of this work and a description of the gland in *Zootermopsis* and *Nasutitermes* will be published elsewhere.

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CYTOLOGY

Auto-Alloploid Nature of *Pennisetum squamulatum* Fresen

CYTOLOGICAL observations carried out in this laboratory on *Pennisetum squamulatum*, an East African[1] grass species, and on its F_1 hybrid[2] with pearl millet (*Pennisetum typhoides* Stapf and Hubb.), have suggested that *P. squamulatum* is an auto-allopolyploid.

Raman, Chandrasekharan and Krishnaswamy[3] reported that the species possessed $2n = 54$ chromosomes and that, during meiosis in the pollen-mother-cells, bivalents and quadrivalents (1 per cell) were formed. We re-examined meiosis in the pollen-mother cells of this species and observed the types of chromosome association which suggested the polyploid nature of this species (Table 1).

Table 1

	Quadrivalents	Trivalents	Bivalents	Univalents
Average frequency per cell	3·14	1·06	17·90	1·91
Range	1–8	0–2	11–23	0–4

Total cells observed : 36

During 1958 the cross, *P. typhoides* × *P. squamulatum*, was attempted on an extensive scale. For this, pollen from a single plant of *P. squamulatum* was dusted on a large number of ears of *P. typhoides* without emasculation. In 1959, 11 plants were raised in the field from the seed set on the male parent and about 600 plants from the seed set on the ears of *P. typhoides* used for crossing. Seven out of these 11 plants of *P. squamulatum* were examined cytologically, and all of them were found to possess

$2n = 54$ chromosomes, thus suggesting that, despite multivalent and univalent formation, the species was fairly balanced in respect of chromosome number. Of the plants raised from the seed set on the crossed ears, all but one were matromorphic. The single hybrid plant possessed $2n = 41$ chromosomes. During meiosis in the pollen-mother-cells of the hybrid 32 out of 36 clearly analysable cells showed 16 bivalents and 9 univalents; the remaining four cells showed 14–15 bivalents and 11–13 univalents per cell.

From the number of chromosomes and from the most frequent mode of chromosome association observed in the hybrid during meiosis it would appear that the hybrid plant arose from the union of an unreduced 14-chromosome *typhoides*-gamete with a normal 27-chromosome *squamulatum*-gamete. It would also appear that, of the 16 bivalents observed in the hybrid, 7 were derived from the *typhoides*-parent and 9 from autosyndetic pairing in the 18 out of the 27-chromosome complement derived from the *squamulatum*-parent. These observations further suggest that *P. squamulatum* ($2n = 54$) is an auto-allopolyploid[4] comprising in its somatic complement a 9-chromosome genome represented in the autotetraploid condition and another 9-chromosome genome in the diploid condition. Supporting evidence for such a view is obtained from the study of meiotic chromosome behaviour in *P. squamulatum*, where as many as 8 quadrivalents, out of the possible 9, were noticed in two cells.

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A Simplified Method for the Study of Chromosomes in Man

Bottura and Ferrari[1] have described recently a rapid technique for the cytological preparation of human chromosomes, which involves intravenous injections of deacetylmethylcolchicine 2 hr. prior to a sternal marrow puncture. The marrow is then treated with hypotonic citrate, hydrolysed and stained. As they rightly point out, there are some advantages in this rapid technique over the short- or long-term tissue culture.

We have been employing similar techniques and have obtained good preparations of human chromosomes from dividing cells in the sternal marrow by giving the patient 1 mgm. of colchicine intravenously 1 hr. before the puncture. The material is then placed in 0·72 per cent w/v sodium citrate for 10 min., fixed in 3 : 1 (ethanol acetic), hydrolysed for 6 min. in *N* hydrochloric acid and stained in orcein (Fig. 1). Satisfactory preparations have also been made without giving the colchicine, relying only on the action of the hypotonic citrate; although, as would be expected, the contraction of the chromosomes is not then so well developed.

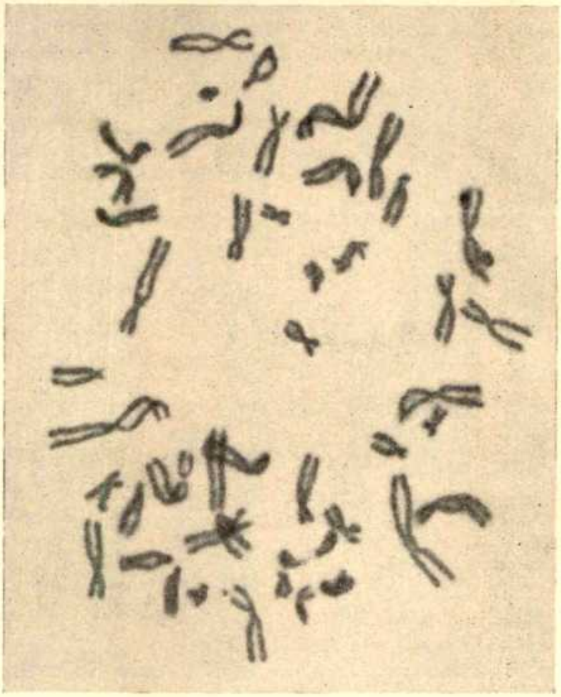

Fig. 1

These simple procedures are extremely useful for the rapid screening of human material. They would suffer perhaps from the disadvantage that they may not detect a chimærical situation. Their outstanding advantage, however, is the establishment of the *in vivo* situation in conditions such as leukæmia, where the results of culture techniques may not be truly representative.

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Use of Induced Rooting in Cytological Studies

The common practice for the study of somatic chromosomes is to cut sections or squash the growing root-tips of a seedling. Occasionally successful preparations of somatic chromosomes have also been made from very young leaves and buds, but still in most cases root-tips continue to be favourite material for the study of somatic chromosomes. Seeds of wild plants, however, unlike those of the cultivated ones, are not always easily available, and this accounts at least in part for the fact that chromosome number in members of the former group have either not been determined or, if determined at all, reported from meiotic studies. This is evident from a cursory examination of the "Chromosome Atlas of Flowering Plants"[1]. The present work was started to find if these difficulties could be overcome by inducing adventitious root formation in isolated shoots and leaves by means of treatment with various growth-promoting substances.

The procedure to induce rooting in some of the species used as given here is more or less the same as that adopted by Gregory and Samantarai[2].

Stem and leaf of *Rauwolfia serpentina* Benth. and *Aralia filicifolia* L. have been treated with synthetic hormones, indole-3-acetic acid and naphthyl acetic acid. Several concentrations of these hormones were tried for treatment. In each concentration used, either stem cutting or the petiole of a leaf was placed for 24 hr. Then after thorough washing in running tap-water for ½ hr., the cutting or the leaves were transferred to specimen tubes containing tap-water which in turn were placed in a humid glass chamber. The materials were washed every 24 hr. in running tap-water and put back in the moist chamber. Indole-3-acetic acid of any concentrations could not induce rooting in *Aralia filicifolia* L., but some roots came out from the stem cutting of *Rauwolfia serpentina* Benth. after 25–30 days from the date of the treatment in 50 p.p.m. On the other hand, stem cuttings and a solitary leaf of the above two species, when treated with naphthyl acetic acid (10 p.p.m. for the solitary leaf and 50 p.p.m. for the stem), induced rooting after only 10 or 12 days. Thus it seems that naphthyl acetic acid is more effective for inducing rooting in these plants than indole-3-acetic acid, which is also evident from the fact that naphthyl acetic acid-treated materials developed copious and healthy roots, instead of solitary roots obtained from indole-3-acetic acid-treated materials. The stem and the leaf for such treatment should not be very old.

Squash preparations of the root-tips of the above species have been made after pre-treatment with 0·002 *M* 8-hydro-oxyquinoline for 3–4 hr. at about 18° C. The root tips were squashed either in 1 per cent propionic orcein or in acetic orcein. Before squashing, the root-tips were stained in a solution of 2 per cent acetic or propionic orcein and *N* hydrochloric acid in a ratio of 9 : 1 for 5 min. at 60° C. This staining procedure of keeping the root-tips for 5 min. at 60° C. in 9 : 1 acetic or propionic orcein and *N* hydrochloric acid has been found to give much better results, that is, uniform staining, than that given by Sharma and Mookerjee[3] in which the root tips are warmed on a burner. It is possibly due to non-uniformity of heating on a burner that the materials sometimes appear as under- or over-stained.

The chromosome counts of the species noted above show $2n = 24$ in *Aralia filicifolia* L., which is the same number as reported by Wanscher[4] and $2n = 20$ in *Rauwolfia serpentina* Benth. Since the somatic chromosome number of *Aralia filicifolia* L., determined here from induced roots, is the same as that reported by Wanscher from normal roots, it appears that growth-promoting substances do not in any way disturb the chromosome number and morphology.

My sincere thanks are due to Prof. R. P. Roy and Dr. J. P. Sinha, Botany Department, Patna University, for their encouragement and help.

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GENETICS

New Recurrent Series in Inbreeding Phenomena

A number of recurrent series have been found in phenomena related to inbreeding. A new series is represented by the sequence of the values of f'_m (the mean coefficient of inbreeding for sex-linked genes[1] in a strictly panmictic population model, as defined by Freire-Maia and Freire-Maia[2]), from sib matings through the subsequent simple full degrees of consanguinity (uncle–niece plus aunt–nephew, $UN + AN$; first cousin, $1C$; first cousin once removed, $1\frac{1}{2}C$; second cousin, $2C$; etc.). This series is as follows: 1/4, 3/16, 5/64, 11/256, 21/1,024, 43/4,096, 85/16,384, . . . (Fig. 1) (cf. Freire-Maia and Freire-Maia[2] and unpublished work, and Freire-Maia and Marçallo[3] for these terms). Knowing two consecutive values, the following value may be calculated by the formula:

$$x'_n = \frac{1}{4}\left(x'_{n-1} + \frac{1}{2}x'_{n-2}\right)$$

The general solution for this equation is:

$$x'_n = \frac{1}{3}\left(\frac{1}{2}\right)^n - \frac{1}{12}\left(-\frac{1}{4}\right)^n \qquad (1)$$

Excluding x_0, which corresponds to sib mating, and x_1, corresponding to $UN + AN$, all the other consanguineous matings (that is, the cousin matings) have their positions in the series given by multiplying their respective abbreviated representation (1, 1½, 2, 2½ . . .) by 2. Third cousins once removed ($3\frac{1}{2}C$) are, for example, the seventh term.

The sequence of the values of f (Wright's coefficient of inbreeding for autosomal genes) for the offspring of the same above-mentioned consanguineous unions also forms a recurrent series (1/4, 1/8, 1/16, 1/32, 1/64 . . .) which is also a geometric progression (Fig. 1). Any one of its terms may be calculated according to the formula:

$$x_n = \frac{1}{4}\left(\frac{1}{2}\right)^n \qquad (2)$$

In these two recurrent series, the limiting value of x'_n and x_n as $n \to \infty$ is 0. This limit is approached in an oscillatory way by x'_n in the first series (cf. formula 1).

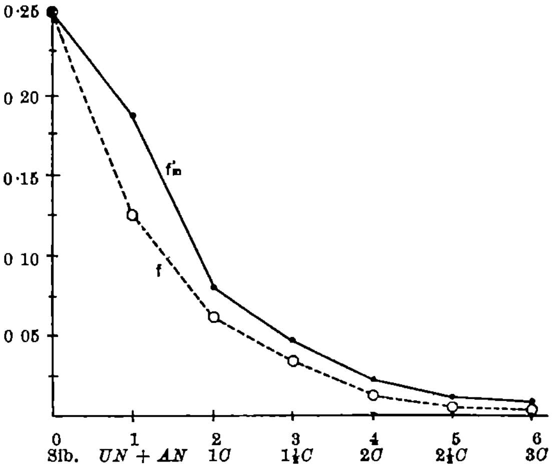

Fig. 1. The first values of f and f'_m

The ratio of formula (1) to formula (2) is :

$$x'_n = \frac{4}{3} - \left[\frac{1}{3}(-1)^n\right]\left(\frac{1}{2}\right)^n$$

which is the general formula for any term of a new recurrent series. The limiting value (4/3) is rapidly approached in an oscillatory way (Fig. 2) similar to that found by Jennings[4] for the equlibrium of sex-linked genes in random mating populations. In cases of distant consanguinity, f'_m may be accepted as being roughly equal to $1 \cdot 33\ f$. The terms of this oscillating series are : 1, 3/2, 5/4, 11/8, 21/16, 43/32, 85/64, 171/128, 341/256, . . . Knowing two consecutive terms :

$$x_n = \frac{1}{2}(x_{n-1} + x_{n-2})$$

The above-mentioned oscillatory approach is due to the fact that the first series is composed of alternating terms of two other series—one for the consanguinity between persons belonging to the same generation (sibs and cousins of the nth degree) and the other for the consanguinity of persons belonging to different generations ($UN + AN$ and cousins of the nth degree once removed) (Fig. 2).

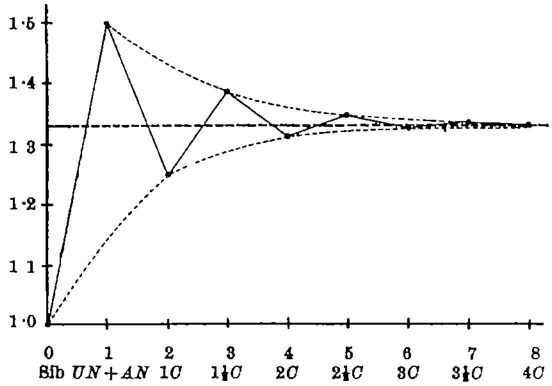

Fig. 2. Oscillatory approach of the ratio f'_m/f to the limiting value 4/3 as $n \rightarrow \infty$

Each term of the series for persons belonging to the same generation may be found as follows :

$$x_n = \frac{5}{16}x_{n-1} - \frac{1}{64}x_{n-2}$$

yielding the general solution :

$$x_n = \frac{1}{3}\left(\frac{1}{4}\right)^n - \frac{1}{12}\left(\frac{1}{16}\right)^n$$

for initial values $x_0 = 1/4$ and $x_1 = 5/64$.

Each term of the series for persons belonging to different generations may be calculated as follows :

$$x_n = \frac{5}{16}x_{n-1} - \frac{1}{64}x_{n-2}$$

yielding the general solution :

$$x_n = \frac{1}{6}\left(\frac{1}{4}\right)^n + \frac{1}{48}\left(\frac{1}{16}\right)^n$$

for initial values $x_0 = 3/16$ and $x_1 = 11/256$.

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Genetic Recombination in Bacteriophage ΦX 174

RECENTLY, Tessman[1,2] and Sinsheimer[3] suggested that the deoxyribonucleic acid of ΦX 174 is one-stranded in contrast to the double-stranded deoxyribonucleic acid of other phages. From this point of view it seemed interesting to investigate genetic recombination in this phage. Tessman[4] achieved recombination in bacteriophage S 13 by stimulation with ultra-violet irradiation of free virus particles. (S 13 is serologically related to ΦX 174 and similar in other properties.) The frequency of recombinants found after ultra-violet irradiation was of the order of 0·1 per cent. In other work[5] without irradiation, no recombination could be detected.

A selective system for host-range recombinants, similar to the negative host-range system in T2 (ref. 6), could be developed in ΦX 174 : *E. coli C* were plated on colour-agar plates (Bresch and Trautner[7]) with an excess of ΦX 174 and a resistant strain C_a was isolated. A host-range mutant of ΦX 174 against C_a was then selected and called *Ha*. By repeating these procedures a second-step bacterial mutant C_{ab} and phage mutant *HaHb* were obtained. A third step resulted in a triple bacterial mutant C_{abc} and a triple phage mutant *HaHbHc*. This *HaHbHc* stock was treated with nitrite, which induces mutations by affecting the nucleic acids[8]. Negative host-range mutants (revertants) appear as turbid plaques on mixed indicator *C* plus C_{abc}.

Fifteen such revertants (numbered 1–15) were collected and crossed to each other and to the one-step (*Ha*) and two-step host-range mutant (*HaHb*). Many of these crosses resulted in recombinants, able to grow on C_{abc}. In Table 1 the recombining crosses are represented by + and the non-recombining by −. The fraction of recombinants was between 1×10^{-4} and 6×10^{-4}. The background of mutation to a C_{abc} lysing type, controlled by the 'self-crosses', was in all cases less than 10^{-7}.

From Table 1 the revertants can be placed in three groups according to whether the back mutation occurred either in *Ha*, *Hb* or *Hc*. Two exceptions (Nos. 2 and 3) seem to show reversions at two sites. A preliminary quantitative evaluation of the recombination data suggests a linear order *HaHbHc*, where the distance *Ha–Hb* is about half the distance *Hb–Hc*.

Table 1 CROSSES BETWEEN REVERTANTS, EITHER YIELDING RECOMBINANTS (+) OR NOT (−)

	8	11	12	1	5	13	14	15	4	6	7	9	10	*HaHb*	*Ha*	3	2
8	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
11	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
12	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
1	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	−	+
5	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	−	+
13	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	−	+
14	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	−	+
15	+	+	+	−	−	−	−	−	+	+	+	+	+	+	−	−	+
4	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
HaHb	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Ha	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
3	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2	−	−	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−

The work is being continued and details will be published later.

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Australian Aborigines and Genetics

PROF. A. A. ABBIE's statements[1] concerning two of my papers[2,3] on the genetics of the Australian aborigines cannot be allowed to pass unanswered. Prof. Abbie admits that he is not a geneticist, yet he throws doubt on genetical methods of racial study, even suggesting that nearly three months in Australia was insufficient time for me to obtain reliable results. He is unwilling to recognize that anatomy can only make *comparisons* between racial types, while genetical methods of family study in crosses give an *analysis* of racial differences and are thus of far greater significance. His thesis appears to be that the aboriginal population of the whole continent is a unit, and that all the evidence which shows the presence of mixed elements must be resisted or denied.

Prof. Abbie makes various comparisons of means and ranges in different local populations, for example, in stature, but he makes the long outmoded assumption that these are mere variations from a mean. He fails to see that measurements are of little value unless there is some understanding of the method of inheritance of the differences involved. Much evidence shows that stature in various races of mankind depends in part on multiple genes which are cumulative in effect, the number of such genes being probably a small one for each racial character. This applies both to stature and hair form.

Those who see the limitations of Prof Abbie's anatomical methods should advocate the genetical approach to race studies; but unfortunately very few, at present, have the necessary combination of genetical and anthropological knowledge and experience.

As regards the Mongolian (better sacral) spot, one would be surprised to find it in the aborigines in view of all their other racial characters, which are mainly archaic Caucasoid except the darker skin colour.

Regarding the dwarf element in certain tribes of Queensland, Prof. Abbie does not mention that my conclusions are a confirmation of those of Tindale and Birdsell[4] that these people are a remnant of a Negritoid type which was pushed down into Tasmania (with a limited amount of crossing with Australian aborigines) when the latter entered Australia from the north and moved south. There are few cases of early racial migration for which the evidence to unprejudiced minds is so unequivocal. Yet Prof. Abbie not only denies it but says that this is "a fair example of ignoring facts to push a hypothesis". He achieves this by referring to the fact, stated by Tindale and Birdsell[4] as well as myself[3], that some of the Queensland dwarfs have a yellowish skin colour. He is evidently unaware of the elementary fact that this is simply due to dilute melanin. The situation is similar regarding hair form, where a few multiple genes for curly hair will account for the dispersal of this character as an occasional occurrence through the continent of Australia.

Regarding head form, Prof. Abbie ignores the extensive genetic results of my work from the study of families of mixed descent (Aborigine × White) in three generations, mainly at Alice Springs and Darwin, which breaks new ground and shows the remarkable genetic nearness of these aboriginals to the archaic Caucasoids of Europe.

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I WISH to make the following comments on Prof. Ruggles Gates's communication.

I freely admit to being an old-fashioned anatomist employing an archaic methodology; this, however, has the minor advantage of dealing in facts, some of them even measurable.

The close association of Australian aborigines with some basic 'Caucasoid' stock has long been widely accepted. I mentioned our failure to find a 'sacral spot' purely in passing, because some observers had previously claimed that it existed. Whether or not that had any relationship to presumed 'Caucasoid' affinities of the aborigines is beside the point.

I apologize to Prof. Ruggles Gates for any seeming discourtesy over his observation that the Queensland aborigines he examined have a yellowish skin, but I must point out that he laid himself open to adverse comment. He cites "a tendency toward yellowish brown skin colour" as characteristic of "Barrineans". I said that Prof. Ruggles Gates knew that in all aborigines the skin fades to a yellowish colour when protected from direct sunlight; this, of course, will occur in the rain forest of north-east Queensland. I am quite well aware that the yellowish colour is probably due to dilute melanin, as I had indicated in an earlier paragraph. But if any aborigine can fade to a yellowish colour, such colouring cannot be considered ethnically distinctive within Australia.

A. A. ABBIE

University of Adelaide.

SOIL SCIENCE

Microbiological Changes in Freshly Moistened Soil

It is well known that when a dried soil is moistened there is a burst of respiratory activity. Drying, in some way, renders a part of the soil organic matter soluble and it has been considered that the decomposition of this fraction by micro-organisms partly accounts for the high respiration-rates recorded. The composition of this soluble material has not been fully determined, but it has been shown to contain appreciable amounts of several amino-acids[1]. In these circumstances the development of a specialized zymogenous population might be expected. Indirect evidence for the existence of such a population has been provided by Chase and Gray[2]. They made a detailed analysis of the respiration of freshly moistened soils and concluded that their results could be best interpreted by assuming that two major microbial populations were involved, namely, one with a low rate of metabolism—the autochthonous population—and the other with a very high rate—the zymogenous population.

Observations made recently on the behaviour of the bacteria in an East African soil (Kikuyu red loam) provides direct evidence for this interpretation. Soil which had been dried was re-moistened to field capacity and maintained at 25° C. Rate of carbon dioxide production and bacterial numbers were recorded at 3-hourly intervals for a period of 36 hr. Carbon dioxide was determined in the macrorespirometer devised by Birch and Friend[3] and bacterial numbers by direct cell counting[4]. Note was taken of the morphology of all cells scored and it was found that the two main cell types (cocci and rods) showed marked differences in their distribution over the 36-hr. period. From Fig. 1 it can be seen that a comparatively small increase in coccoid forms occurred early, their numbers then remaining at a fairly steady value. Rods, on the other hand, showed no increase until after 12 hr., when their numbers increased rapidly to a maximum at 24 hr.

It will be recalled that Winogradsky[5] considered the autochthonous flora of the soil to be composed almost exclusively of cocci and very short rods, whereas the zymogenous flora comprised spore-formers and long rods. The present results can be interpreted as showing that the rods constitute the zymogenous part of the population and, in fact, are responsible for the greater part of the flush of carbon dioxide. Although numerically they do not at any time constitute more than 50 per cent of the total population, their size in comparison with the cocci is such that they must, during their brief period of growth, constitute the greater part of the active cellular material in the soil. Moreover, it is well established that maximum physiological activity occurs during the lag and early log phase in the growth of a bacterial culture and it is clear that in the present instance maximum carbon dioxide production coincides with this part of the growth curve of rods rather than with the point of maximum number.

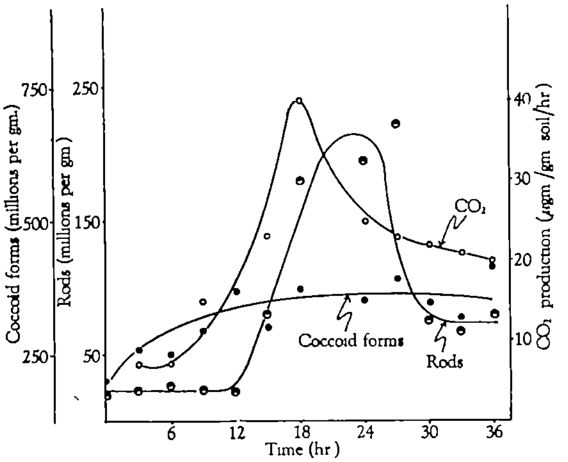

Fig. 1. Carbon dioxide production (○) and development of bacterial populations in moistened soil. ●, Coccoid forms; ⊖, rods

This work, which is being continued and will be reported more fully elsewhere, was undertaken when one of us (E. G.) was in receipt of a travelling grant from the Colonial Office.

E. Griffiths

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H. F. Birch

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[1] Stevenson, I. L., *Plant and Soil*, 8, 170 (1956).

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[3] Birch, H. F., and Friend, M. T., *Nature*, 178, 500 (1956).

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MISCELLANY

Letter from Erasmus Darwin to Jonas Dryander

The following extract from a letter of Erasmus Darwin to Jonas Dryander seems worth noting. The letter is in a small collection in the Department of Botany, British Museum (Natural History) labelled "Dryander Correspondence".

"Derby, May 21–87

. . . I must now beg another favor of you, which is to give your suffrage to my Son, Dr. Robt. W. Darwin, whom I wish to be a member of the Royal Society, which might add new spirit to his industry in philosophical pursuits ; & should be much obliged to you to present my best compl. to Dr. Blagden, when you see him, & ask for my Son the same favor from him. Mr. Ch. Greville has spoken to Sr. Joseph Banks in his favor, whom I should otherwise have troubled with a letter on this account. Doctor Robt. Darwin is settled in Shrewsbury, where he meets with very great encouragement, having been concern'd for near fifty patients in the first six months ; & I dare say will make a very useful member of the Society."

Robert Darwin "began to practise before he was twenty-one years old" (Charles Darwin, *The Autobiography*).

John Ramsbottom

Richmond,
Surrey.

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 6

University of London (in the Assembly Hall, Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Mr. R. W. Young · "The University and the Grammar School".*

British Society for the History of Science (in the London Planetarium, Marylebone Road, London, N.W.1), at 5.30 p.m.—Mr. Leonard Clarke : "Greek Astronomy and Its Debt to the Babylonians".

Society of Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. W. E. Humphrey : Presidential Address.

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Dr. James Taylor : "The Modern Chemical Industry in Great Britain". (Last of three Cantor Lectures.)

Society of Chemical Industry, London Section (joint meeting with the London Section of the Royal Institute of Chemistry, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. H. H. Chambers : "Fuel Cells".

Society of Instrument Technology, Data Processing Section (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Prof. J. E. Parton and Mr. A. Rose : "The Universal Decision Element".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. J. Ward-Perkins : "Etruria's Past".

Tuesday, February 7

Society of Chemical Industry, Agriculture Group (at 14 Belgrave Square, London, S.W.1), at 10.30 a.m.—Meeting on "Alternative Nitrogen Fertilizers".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. W. G. Chaloner : "Wandering Continents and Fixed Ideas—a Discussion of the Evidence for Continental Movement".*

British Institution of Radio Engineers (joint meeting with the Electronics Group of the Institute of Physics, at the Institute of Physics, 47 Belgrave Square, London, S.W.1), at 2.15 p.m.—Meeting on "Tunnel Diodes".

British School of Athens (at the Royal Geographical Society· 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Sinclair Hood : "Excavations at Knossos (Crete), 1960".

Institution of Electrical Engineers, Measurement and Electronics Sections (at Savoy Place, London, W.C.2), at 5.30 p.m. —Mr. E. M. Bradley : "Magnetic Properties of Thin Films for Computing Devices".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. R. Knox : "Contrasting Patterns of Drug-Resistance in Tubercle Bacilli and Staphylococci".* (Eighth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Royal Institute of Chemistry (joint meeting with the Sir John Cass College Chemical Society, at the Sir John Cass College, Jewry Street, London, E.C.3), at 6.30 p.m.—Prof. B. C. L. Weedon : "Organic Reactions in Strong Alkalis".

Wednesday, February 8

Institute of Physics and the Physical Society, Low Temperature Group (at 47 Belgrave Square, London, S.W.1), at 2.30 p.m.—Meeting on "Ultrasonic Absorption and Attenuation in Metals at Low Temperature".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. Rodney Perry : "The Timber Industry".

Eugenics Society (at Manson House, 26 Portland Place, London, W.1), at 5 p.m.—Sir Robert Platt, Bt. : "Inherited Disease in Man" (The Galton Lecture.)

British Institution of Radio Engineers, Computer Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. G. Emery . "Perseus, a Medium-scale Data Processing System".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. F. G. E. Pautard : "The Molecular Biology of Calcified Tissues".* (Second of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)

Women's Engineering Society, London Branch (at Hope House, 45 Great Peter Street, Westminster, London, S.W.1), at 7 p.m.—Mr. L. J. Carter : "Space Exploration".

Thursday, February 9

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. R. A. French : "Russian Rivers in the Service of Man".*

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. Latham and Mr. B. J. Mason : "Electric Charge Transfer Associated with Temperature Gradients in Ice" and "Generation of Electric Charge Associated with the Formation of Soft Hail in Thunder-clouds".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. P. J. Grubb : "Alpine Plant Communities in Southern Jugoslavia".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. H. Collier : "Trachoma and Inclusion Blennorrhoea".* (Ninth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.

Chemical Society (at Burlington House, Piccadilly, London, W.1 at 7.30 p.m.—Meeting for the reading of Original Papers.

Thursday, February 9—Monday, February 27

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7)—Greenland—an Exhibition.

Friday, February 10

Association of Applied Biologists (at the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.40 a.m.—Papers on "Recent Research on Plant Virus Diseases".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Sir George Sutherland, F.R.S. : "Diamonds".

Saturday, February 11

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. William Fagg · "The Art History of Benin, Nigeria".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned

Assistant Lecturer (preferably skilled in or possessing a knowledge of mass spectroscopy as applied to chemical problems) in Physical Chemistry—The Registrar, The University, Leeds 2 (February 14).

Imperial Chemical Industries Fellows for Research in various branches of Chemistry, Chemical Engineering, Engineering, Technology, Physics, and allied subjects—The Registrar, The University, Manchester 13 (February 14).

Turner and Newall Research Fellows in Engineering, Inorganic Chemistry, Physics and allied subjects—The Registrar, The University, Manchester 13 (February 14).

Senior Assistant Observer (with a good honours degree in mathematics, physics or astronomy, and some experience of research in astrophysics)—The Director, Observatories, The University, Madingley Road, Cambridge (February 15).

Technical Officer (with a degree in physics or engineering and a good practical knowledge of electronics), for research into photoelectric photometry by pulse counting techniques—The Director, Observatories, The University, Madingley Road, Cambridge (February 15).

Demonstrator (with a degree in science or agricultural science) in Agronomy at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 15).

Lecturer (physicist, preferably with an interest in observational science) in the Department of Meteorology—Professor of Meteorology, Imperial College of Science and Technology, London, S.W.7 (February 20)

Assistant Lecturers (2) in Biochemistry—The Registrar, Manchester College of Science and Technology, Manchester 1 (February 25).

Assistant Lecturer or Lecturer (graduate in microbiology, bacteriology, botany, biochemistry or cognate fields) in Microbiology—The Registrar (Room 22, O.R.B.), The University, Reading (February 28).

Lecturer or Assistant Lecturer in the Department of Mathematics at Makerere College (The University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

Scientific Officer (with a first- or second-class honours degree in mathematics with statistics, or a diploma in statistics) in the Statistics Section for duties mainly concerned with the design and analysis of field experiments—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (February 28).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry or Physics—The Registrar, University College, Dublin (March 7).

Lecturer (with an honours degree in botany or agricultural botany, postgraduate research and training in plant pathology, and preferably an interest in host-parasite relations); and an Assistant (with an honours degree in botany or agricultural botany, training in plant pathology, and preferably an interest in virus diseases) in the Department of Botany—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (March 11).

Lecturer or Assistant Lecturer in Statistics—Head of the Department of Mathematics, Imperial College of Science and Technology, South Kensington, London, S.W.7 (March 24).

Assistant Keeper in the Department of Botany—The Secretary, National Museum of Wales, Cardiff (April 1).

Research Students (geology graduates) in Geochemical Prospecting—Dr. Webb, Geology Department, Imperial College of Science and Technology, South Kensington, London, S.W.7 (June 1).

Agricultural Chemist (with a good honours degree in agricultural chemistry or chemistry with at least two years postgraduate research experience in agricultural chemistry) in Kenya, to take charge of research projects into soil and plant nutrition problems of coffee—

The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/7/050.

ANIMAL GENETICIST, to undertake research in quantitative genetics with the ultimate objective of obtaining basic information needed for an understanding of inheritance of economic traits in livestock and poultry; a FOREST BIOLOGIST (PLANT or FOREST PATHOLOGIST), to design, conduct and supervise research in diseases of forest nursery stock; and a QUANTITATIVE GENETICIST (MATHEMATICIAN or STATISTICIAN), to conduct research into methods appropriate for dealing with problems in quantitative genetics, with the Canadian Department of Agriculture—Civil Service Commission of Canada, Ottawa, Canada, quoting Ref. 61–2201.

EDITORIAL ASSISTANT (with a university degree in science or technology, and preferably at least two years postgraduate experience in science or industry)—The Manager, *Nature* Office, Macmillan and Co., Ltd., St. Martin's Street, London, W.C.2.

GRADUATE ASSISTANTS (qualified to pursue M.Sc. and Ph.D. work in chemistry)—The Chairman, Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.

HEAD (with good academic qualifications in science, and preferably teaching, industrial and administrative experience) OF THE DEPARTMENT OF SCIENCE—The Principal, Cambridgeshire Technical College and School of Art, Collier Road, Cambridge.

PHYSICISTS, ENGINEERS and MATHEMATICIANS, Scientific Officer or Senior Scientific Officer grade (with a first- or second-class honours degree or equivalent in physics, electrical engineering or mathematics, and at least three years postgraduate experience for Senior Scientific Officer grade) at the Radio Research Station, D.S.I.R., Ditton Park, Slough, to investigate the upper atmosphere and the space between the Earth and the Sun, with particular reference to the propagation of radio waves through these regions, and associated problems of terrestrial and space communications—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.581/0A.

RESEARCH WORKER (BIOCHEMIST or CHEMICAL PHARMACOLOGIST, preferably with Ph.D. or equivalent experience), to investigate the biochemical actions of drugs currently used in the treatment of mental illness—The Director of Clinical Research, Crichton Royal, Dumfries.

SENIOR LECTURER or LECTURER (with high academic qualifications, preferably in metallurgy, coupled with practical experience of the techniques and the engineering utilization of welding) IN WELDING TECHNOLOGY—The Recorder, The College of Aeronautics, Cranfield, Bletchley, Bucks.

SENIOR TECHNICIAN FOR WORK IN THE BACTERIOLOGICAL DIVISION at Connaught Hospital, Walthamstow, London, E.17—The Secretary, Forest Group H.M.C., Langthorne Road, London, E.11.

SOIL SURVEYOR (age 26–40, with a degree in natural science or geography, with two years' postgraduate training or experience in soil survey work) in Sarawak, to carry out soil surveys—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD. 63/24/07.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

British Commonwealth Collections of Micro-Organisms. Directory of Collections and List of Species maintained in Australia. Pp. v+45. 8*s*. net. Directory of Collections and List of Species maintained in Canada. Pp. iv+48. 9*s*. net. Directory of Collections and List of Species maintained in India. Pp. iv+86. 6*s*. net. Directory of Collections and List of Species maintained in New Zealand. Pp. iv+16. 3*s*. net. Directory of Collections and List of Species maintained in the United Kingdom, Ghana and Trinidad. Pp. iv+70. 12*s*. 6*d*. net. (London: H.M. Stationery Office, 1960.) [711

Trans-Antarctic Expedition, 1955–1958. Scientific Reports. No. 2: A Gravity Traverse of Antarctica. By J. G. D. Pratt. Pp. 22. 12*s*. 6*d*. net. No. 3: Seismic Soundings Across Antarctica. By J. G. D. Pratt. Pp. 69. 30*s*. net. No. 4: Tides at Shackleton, Weddell Sea. By J. G. D. Pratt. Pp. 21+2 plates. 12*s*. 6*d*. net. (London: Trans-Antarctic Expedition Committee, 22 Gayfere Street, S.W.1, 1960.) [711

Discovery Reports, Vol. 30, pp. 301–408, plates 7–28, August, 1960: Studies on *Physalia physalis* (L.). Part 1: Natural History and Morphology. By A. K. Totton. Part 2: Behaviour and Histology. By G. O. Mackie. (Cambridge: at the University Press, 1960.) [711

Institution of Electrical Engineers. The Training of Graduates: a Report of the Joint Committee on Practical Training in the Electrical Engineering Industry. Pp. 34. (London: Institution of Electrical Engineers, 1960.) 2*s*. [811

Department of Scientific and Industrial Research: Geological Survey and Museum. The Origin and Evolution of Coal. By Dr. P. J. Adams. Pp. i+17. (London: H.M. Stationery Office, 1960.) 1*s*. 9*d*. net. [811

Tobacco Manufacturers' Standing Committee. Report for the year ended 31st May, 1960. Pp. 11. (London: Tobacco Manufacturers' Standing Committee, 1960.) [811

Endemic Goitre: World Distribution and Incidence—Select Bibliography. Second edition. Pp. iii+136. (London: Chilean Iodine Educational Bureau, 1960.) [911

Current Problems in the Understanding of Personal Responsibility: a Bibliography selected from the fields of Psychology, Sociology, Ethics and Theology. List A: Pp. 11. 1*s*. List B: Pp. 11. 2*s*. (London: Church Information Office, Church House, S.W.1, 1960.) [911

Tube Investments, Ltd. Extended Surface Tube. Pp. 28. (London: Tube Investments, Ltd., 1960.) [911

A National Fuel and Power Policy: a Policy Statement by the Association of Scientific Workers. Pp. 20. (London: Association of Scientific Workers, 15 Half Moon Street, W.1, 1960.) [1011

Department of Scientific and Industrial Research: Building Research Station. National Building Studies Research Paper No. 28: Studies in Composite Construction. Part 3: Tests on the New Government Offices, Whitehall Gardens. By R. J. Mainstone. Pp. vi+54+8 plates. (London: H.M. Stationery Office, 1960.) 8*s*. 6*d*. net. [1011

C. F. Casella and Co., Ltd. One Hundred and Fifty Years of Scientific Instrumentation, 1810–1960. Pp. 12. (London: C. F. Casella and Co., Ltd., 1960.) [1011

Report from the Select Committee on Nationalised Industries, together with the Proceedings of the Committee, Minutes of Evidence and Appendices—British Railways. Pp. cvi+505. (254–1.) (London: H.M. Stationery Office, 1960.) 22*s*. 6*d*. net. [1011

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Other Countries

Canada: Department of Northern Affairs and National Resources National Museum of Canada. Bulletin No. 169: The Birds of West-Central Ellesmere Island and Adjacent Areas. By David F. Parmelee and S. D. MacDonald. Pp. iv+103 (10 plates). 1.50 dollars. Bulletin No. 170: An Archaeological Analysis of Eastern Grent Land, Ellesmere Island, Northwest Territories. By M. S. Maxwell. Pp. iv+109 (17 plates). 1.50 dollars. (Ottawa: Queen's Printer, 1960.) [711

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Smithsonian Institution, Washington, D.C. Proceedings of the United States National Museum. No. 3436: Revision of the Milliped Genus *Cherokia* (Polydesmida: Xystodesmidæ). By Richard L. Hoffman. Pp. 227–264. No. 3437: Re-examination of Species of Protura described by H. E. Ewing. By F. Bonet and S. L. Tuxen, Pp. 265–305. No. 3438: Studies in Neotropical Mallophaga. 17: A New Family (Trochiliphagidæ) and a New Genus of the Lice of Hummingbirds. By M. A. Carriker, Jr. Pp. 307–342. No. 3439: The Pelagic Amphipod Genus *Parathemisto* (Hyperiidea: Hyperiidæ) in the North Pacific and Adjacent Arctic Ocean. By Thomas E. Bowman. Pp. 343–392. No. 3442: Descriptions on New Bats from Panama. By Charles O. Handley, Jr. Pp. 459–479. No. 3444: Noctuid Moths of the Scopulepes Group of *Hemeroplanis* Hubner. By E. L. Todd. Pp. 505–515. (Washington, D.C.: Government Printing Office, 1960.) [711

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Comité International de la Détergence: Commission Internationale de Terminologie. Classement des Agents de Surface. (Classification of Surface Agents.) Pp. 15. (Paris: Comité International de la Détergence, 70 Champs-Élysées, 1960.) 1.40 NF. [1011

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CONTROL OF UNIVERSITY BUILDING DEVELOPMENT

ON December 20, the Prime Minister announced in the House of Commons that Lord Robbins, *professor of economics in the University of London*, had accepted the chairmanship of the provisional committee to inquire into higher education in Britain. The full composition of the Committee has not been announced, but its terms of reference were given as follows:

"To review the pattern of full-time higher education in Great Britain and in the light of national needs and resources to advise Her Majesty's Government on what principles its long-term development should be based. In particular, to advise, in the light of these principles, whether there should be any changes in that pattern, whether any new types of institution are desirable and whether any modifications should be made in the present arrangements for planning and co-ordinating the development of the various types of institution."

The long-term development of universities, colleges of advanced technology, certain other colleges of further education and teacher-training colleges, Mr. Macmillan added, would be within these terms of reference. The Government, he said, is trying to get a very broad representation on the Committee and, by doing so, to deal with all the possible groups that should be considered.

These terms of reference clearly permit the Committee to consider the question of university expansion not in isolation but in relation to the educational system as a whole. It could, for example, comment on the merits or demerits of dealing with higher technological education separately from university education, and could probe into the question of relations between the University Grants Committee and the Ministry of Education. It is true that the Robbins Committee is not expected to publish its findings for two or three years, and the University Grants Committee, which is already discussing with the Treasury the increase in non-recurrent grants entailed by university expansion, is expected by this autumn to submit to the Chancellor of the Exchequer its estimate of what the universities will need from Parliament during the next quinquennium—1962–67. The limit to that immediate expansion is likely to be set by the availability of academic staff and capital resources, and the Robbins report should be available when the questions of how far and where the university system should be further expanded, what are its purposes and what should be its relation qualitatively and quantitatively to the educational system of the country as a whole, require decision. Moreover, in the meantime there may be available a good deal of guidance on such matters as curricula, university entrance and the nation's demands for particular categories of trained man-power and woman-power, and supporting auxiliaries.

Two matters that bear closely on the expansion or modification of the university system in relation to national needs have recently received some attention. The first relates to the financial aspect of expansion. When the report of the Gater Committee, which inquired into the methods used by the universities in contracting and in recording and controlling expenditure from non-recurrent grants, was published in 1956, it was contemplated that the application by the universities of the Committee's recommendations would be reviewed at a later date, and in 1959 the University Grants Committee invited Sir Arthur Rucker to undertake this review. Sir Arthur's report has now been published*, and confirms that, with minor exceptions, the procedures recommended by the Gater Committee are being followed. The University Grants Committee accepts Sir Arthur Rucker's recommendations regarding these exceptions, as well as recommendations for further amendments in the procedure both of the universities and of the University Grants Committee itself.

Sir Arthur Rucker emphasizes first both the magnitude of the building programme of the universities seen as a whole and the success already being achieved. As already announced, up to the end of 1963 the programme provides for an increase in student population from 85,000 in 1955–56 to 135,000 in the second half of the 1960's, and if the further expansion to 170,000 already under discussion is realized, the student population of the universities will have been doubled within a relatively short period of years. On January 25, the Chancellor of the Exchequer, in a written answer in the House of Commons, indicated that further expansion had been approved. He had now authorized the University Grants Committee, he stated, to increase the Government-financed university building programmes starting in the calendar years 1962 and 1963 from the present £15 million to £25 million in each year, and for the years 1964 and 1965 he had authorized the Committee to invite the universities to make building plans on a basis of starts of £30 million in each year. The rate at which these plans can be executed must depend on the general state of the national economy and on such factors as building costs; but Mr. Selwyn Lloyd did not anticipate that the figure ultimately authorized would be less than £25 million a year, and he hoped it would be up to £30 million in each year. This large addition to the resources of the universities should, he added, assist materially in the practical task of accommodating the increased numbers of students who would be coming forward in the second half of this decade, and it would be compatible with further development after 1965 to expand the university population to about 170,000 by the early 1970's.

Controversy, occasionally acute, over the merits of individual university buildings, should not be allowed to obscure the truth that a period of great university expansion is in progress in Britain, and that buildings well suited to its needs will have been provided when the programme of grant-aided buildings has been completed. The real questions are rather where such university expansion should take place, the extent to which such expansion is desirable, particularly in relation to the simultaneous expansion of technological education generally, and to the training of the vastly greater numbers of technicians and craftsmen who are essential if effective use is to be made of the services of the scientists, technologists and other highly trained products of the universities.

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* University Grants Committee. Methods used by Universities of Contracting and of Recording and Controlling Expenditure. Pp. 28. (Cmnd. 1235.) (London: H.M. Stationery Office, 1960.) 1s. 6d. net.

All the universities use the Gater Report as their guide in defining the procedures to be used in carrying out their building programme, and for the most part the procedures recommended were already in force. Where this was not so, they have, with a few minor exceptions, been adopted and remain in use. Like the Gater Committee, Sir Arthur Rucker found no evidence that grants-in-aid of 90–100 per cent weakened the sense of financial responsibility on the part of the universities; but there are naturally differences of opinion on the standards and costs appropriate to particular buildings or types of building. It is, however, not always easy to decide the extent to which non-recurrent grants for buildings can or should be supplemented by universities from their own resources. The University Grants Committee has already emphasized the value and importance to university life of the generous gifts which universities receive from foundations, firms and individuals, more particularly as a proof of public confidence in the work which the universities are doing in advancing scientific knowledge and providing the trained man-power on which the future of Great Britain depends; and Sir Arthur points out that, if such gifts are to be encouraged, universities must have a reasonable degree of freedom to spend them in the way which they consider most useful.

Some expenditure and some embarrassment in planning would have been avoided, he notes, if the present targets for expansion could have been fixed at the beginning of the building programme instead of by later increases; but now that all universities have established control procedures on the lines recommended by the Gater Committee, Sir Arthur considers it unnecessary for the University Grants Committee to review in detail the expenditures under minor works allocation: such expenditure should be left to the discretion of the university. Again, he emphasizes the need for great care in examining comparisons of costs, as the costs of university buildings, even when intended for similar purposes, vary widely; and in his view it is in the interest of economy that every reasonable opportunity should be taken to acquire land likely to be needed for development, even when no immediate use is in prospect. An Architect's Division with professional staff has already been created in the office of the University Grants Committee, and with its aid the Committee is seeking to collect information and statistics on all aspects of university building, to examine space requirements and to analyse costs. A beginning has been made with the definition of space standards and limits of costs for various types of university building.

Apart from these general observations, Sir Arthur Rucker is concerned mainly with the application of the recommendations of the Gater Committee. Of even greater interest, however, are the comments which he makes on developments since that Committee reported, and the conclusions and recommendations arising out of those comments. The great expansion of the building programme makes it even more essential that building procedures should provide effective safeguards against waste, extravagance or other abuse; whereas in 1954–55 grants paid for building works amounted to £4,674,545 and for professional fees to £306,242, in 1959–60 the corresponding figures were £13,693,621 and £1,959,775. The Chancellor's statement on January 25 increased this figure of £15 million to £25 million in 1962 and 1963 and probably to £30 million in the following two years. Reference has already been made to the way in which the establishment of an Architect's Division under the University Grants Committee is assisting the collection and analysis of information and statistics relating to university buildings, and the definition of cost limits and space standards; and Sir Arthur's inquiry has confirmed the desirability of defining, so far as this is possible, the appropriate limit of cost for a university building at a very early stage in planning. The special requirements of university buildings must nevertheless not be ignored, and procedures for defining limits of cost must be sufficiently flexible to take account of those requirements.

There are four stages in the present procedures of the University Grants Committee for the approval of building grants, and Sir Arthur recommends that they should be so timed that the second stage—submission of Schedule I—takes place two and a half to three years in advance of the date of giving out to tender. Since the universities were informed in 1958 of the buildings which they could expect to start in each of the years 1960–63, this should now be practicable. He also considers that in so far as it is found possible to define cost limits for university buildings, it should be feasible for the University Grants Committee to simplify the present procedures and normally to dispense with much of the detailed examination of plans and estimates. At present, limits of cost are defined for halls of residence and, in part, for scientific buildings; but particularly in respect of scientific buildings, Sir Arthur again stresses the need for flexibility and taking special circumstances and varying needs into account at all stages of planning.

Sir Arthur Rucker was not concerned with standards themselves, but only with the procedures for their definition and application; but he is concerned to emphasize that the procedures under which building grants are approved by the University Grants Committee must be devised and applied in close and constant consultation with the universities. This essential consultation would be facilitated, he suggests, if the University Grants Committee were to appoint a Buildings Sub-Committee with membership drawn mainly from the universities. Whatever the arrangements for consultation may be, it is essential that there should be a clear understanding between the members and officers of the University Grants Committee and the universities and their officers at all levels of staffing. If procedures for the control of grant-aided expenditure are to be successful, the present excellent and harmonious relations between the University Grants Committee and the universities must be maintained.

With these concluding words Sir Arthur Rucker endorses, like the Gater Committee, the essential soundness of the present procedures for controlling the expenditure of these large sums of public money, and also, like that Committee, firmly rejects the attack on the system which the Public Accounts Committee maintained for more than a decade. While, however, it is to be hoped that the Public Accounts Committee will not re-open that issue, this further tribute to university administration indicates the outstanding importance of efficient government of universities at the present time, and further, the necessity for securing public understanding of the work which universities are doing and confidence in the ability with which the universities are meeting the demands made upon them. It is clearly within the terms of reference of the Robbins Committee to inquire into the adequacy of the present pattern of university government if it thought fit; but on this second issue, it must suffice to observe here that, while the present arrangements for university administration have come so well out of the searching inquiry to which the Gater Committee and Sir Arthur Rucker have subjected them on the financial side, it is no less important that these arrangements should be adequate to meet the demands which university expansion in Britain is making on them in many other ways.

A UNIFIED TREATMENT OF MODERN OPTICAL THEORY

Principles of Optics

Electromagnetic Theory of Propagation, Interference and Diffraction of Light. By Prof. Max Born and Prof. Emil Wolf. With contributions by A. B. Bhatia, P. C. Clemmow, D. Gabor, A. R. Stokes, A. M. Taylor, P. A. Wayman and W. L. Wilcock. Pp. xxvi+803. (London and New York: Pergamon Press, 1959.) 120*s*. net.

It is quite impossible to do justice to a work of such depth and scope as *Principles of Optics* within the confines of an ordinary review; even to list the contents would require several pages. A few general remarks will therefore be made in the hope of tempting any reader who has not already done so to look at a copy for himself as soon as possible.

Perhaps the most striking impression that one obtains is of completeness. The scope is best described in the words of the authors' preface: ". . . our discussion is restricted to those optical phenomena which may be treated in terms of Maxwell's phenomenological theory. This includes all situations in which the atomistic structure of matter plays no decisive part. The connection with atomic physics, quantum mechanics and physiology is indicated only by short references wherever necessary." Within this framework it would be difficult to find any aspect of modern optical knowledge that is not covered. But the impression of completeness extends to the treatment as well as to the subject-matter. Compare for a moment the following three brief extracts. On page 111 the equation:

$$(\text{grad}\ \mathcal{S})^2 = n^2$$

is derived, n being the refractive index and $\mathcal{S}$ the eikonal, followed by the statement "it is the basic equation of geometrical optics". On p. 163 we read "When the (two thin) lenses are in contact equation ... may be written in the form $P = P_1 + P_2$ so that the power of the combination is then simply equal to the sum of the powers of the two lenses". Finally, on p. 249 we read "In a photographic enlarger ($\varphi \sim 20°$), large sources (frosted bulbs or cold-cathode tubes) often provide reasonably efficient illumination. In a cinematograph projector ($\varphi \sim 5°$) requiring maxium brightness, an electric filament is suitable". It will be seen that the most advanced mathematical treatment, the familiar working formulæ and the practical implications all find a place.

Principles of Optics is valuable as a work of reference; but it may be that its value in showing the interdependence and essential unity of the different approaches may be even greater. Honours students in physics will find here the often-missing links between theoretical and experimental studies. It is interesting to note that even in some of the most mathematical sections the style is clear and vivid. In the early stage of the development from Maxwell's equations we read "Within this layer construct a small near-cylinder bounded by a stockade of normals to T, roofed and floored by small areas δA, . . .". The diagram is almost unnecessary.

Students should be warned, however, that this is emphatically a book for studying rather than reading. The plan of each section is to begin with the most general theory and proceed later to particular practical cases. A certain amount of searching may therefore be necessary to find some of the more familiar material. For example, the chapter entitled "Foundations of Geometrical Optics" begins with the derivation of the eikonal equation already mentioned and we do not meet Snell's law and the elementary laws of reflexion until seventeen pages later.

There have been so many text-books on optics that it is quite thrilling to find so much that is new in both presentation and material in one volume. Its only disturbing feature is that after spending even a very short time in study one is made to realize the vast depth of the subject and the shallowness of one's own understanding of it.

C. A. Taylor

THE FOURTH INTERNATIONAL CONGRESS OF BIOCHEMISTRY

Proceedings of the Fourth International Congress of Biochemistry

Vienna, 1–6 September, 1958. Vols. 1–15. (London and New York: Pergamon Press, 1959–1960.) £48 net complete. (Separate volumes range in price, according to size, from 63*s*. to 100*s*.)

THE First International Congress of Biochemistry was organized on the initiative of the Biochemical Society, and was held in Cambridge eleven years ago. There were about 1,700 members and the main publication, apart from a greatly treasured book on Gowland Hopkins, was a volume of abstracts of communications which would almost fit into the pocket. In 1958, the Fourth International Congress was held in Vienna. More than 5,000 attended and the publications of this Congress, now complete, amount to no less than fifteen substantial volumes—well over 4,000 pages in all. It is astonishing that this large bulk of published material should have emanated from the proceedings of a meeting lasting only six days. It is a reminder of the organizing efficiency and hard work of Prof. Hoffmann-Ostenhof (who has also acted as general editor of these volumes) and his colleagues in Vienna, who made it possible for so many communications to be given in so short a time. This meant, of course, that members frequently had to make a choice from among several communications which were being given simultaneously. Now, with the publication of these volumes, they can read what happened at the meetings they had to miss.

The first twelve volumes are devoted to the symposia. It should be stressed that the pre-prints which were made available at the Congress are no substitute for these volumes, which contain revised and much more complete accounts of the meetings. Moreover, they give some account of the discussion which took place. The subjects covered (with volume numbers and editors in parentheses) are the following: (1) carbohydrate chemistry of substances of biological interest (M. L. Wolfrom); (2) biochemistry of wood (K. Kratzl and G. Billek); (3) biochemistry of the central nervous system (F. Brucke); (4) steroids (E. Mosettig); (5) antibiotics (K. H. Spitzy and R. Brunner); (6) biochemistry of morphogenesis (W. J. Nickerson); (7) biochemistry of viruses (E. Broda and W. Frisch-Niggemeyer); (8) proteins (H. Neurath and H. Tuppy); (9) physical chemistry of high polymers of biological interest (O. Kratky); (10) blood-clotting factors (E. Deutsch); (11) vitamin metabolism (H. Molitor and W. Umbreit); (12) biochemistry of insects (L. Levenbook).

The colloquia, dealing with topical questions in biochemistry, are given in Volume 13. They include: sulphur metabolism (edited by K. S. Dodgson); purine and pyrimidine biosynthesis (edited by H. Chantrenne); oxygenating enzymes (edited by O. Hayaishi); metabolism of C_1-compounds in micro-organisms (edited by Sir Hans Krebs); biosynthetic mechanisms in higher plants (edited by P. K. Stumpf); and growth chemistry of bacteria (edited by G. Toennies and E. E. Snell). Volume 14 (edited by W. Auerswald and O. Hoffmann-Ostenhof) contains the transactions of the plenary sessions and includes the invitation lectures of the Congress. Prof. E. Chargaff, of New York, lectured on the chemistry of heredity, and Prof. E. Braunstein, of Moscow, on the chemical integration of nitrogen metabolism. Volume 15 consists of abstracts of the sectional papers together with a subject index of the symposia and colloquia, ranging from 'abietic acid' to 'zymosterol'.

The printing, illustrations and binding of these volumes are of high quality. Presumably, most biochemists will have to look to their librarians rather than to themselves to provide the complete set, but fortunately, individual volumes can be bought separately. Whether congress publication can go on increasing in bulk at its present rate, or even be maintained at its present level, is another matter. It might be argued that the amount of material and the cost of its publication are becoming unmanageably large and that, in any event, much of the work will be published in more definitive form in the regular journals. However that may be, to those who were there, these volumes will bring back memories of a week of friendly hospitality in beautiful surroundings—memories, not only of scientific discussions, but also of Mozart's 'Magic Flute' at the Vienna State Opera, and of the first sight of Melk Abbey. All biochemists owe a debt to the organizers of this Congress, and to those who worked afterwards to produce this most impressive permanent record of the meeting.

D. C. HARRISON

PROGRESS IN ALKALOID CHEMISTRY

The Alkaloids

Chemistry and Physiology. Edited by R. H. F. Manske. Vol. 6 (Supplement to Volumes 1 and 2). Pp. xii+442. 14 dollars. Vol. 7: Supplement to Volumes 2, 3, 4, and 5. Pp. xiii+558. 17 dollars. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.)

THE arrangement of these volumes follows that of the five original volumes of "Manske-Holmes" which appeared during the period 1949–55, and which these are designed to supplement. The relation of their chapters and sections to the chapters of the original work is carefully detailed, and the numbering in the bibliographies continues from the numbers previously reached. The authorities from Canada, Czechoslovakia, Germany, Hungary, Switzerland, the United Kingdom and the United States are those who contributed the chemical sections of the original, and, like the editor, have lost nothing of their clarity and comprehensive grasp.

The bibliographies may generally be taken to cover as far as 1957, with addenda in one or two cases to 1958. The two volumes may therefore be regarded as an exhaustive review of progress during something less than a decade in alkaloid chemistry.

There is no systematic supplementing of the nine chapters in Volume 5, by specialists on particular pharmacological aspects—among which, even a short time ago, 'tranquillizer' effects found no place—but this omission is to some extent made good by summaries of relevant pharmacological work included in many chapters of the supplement. An attractive example may be noted in section 5 of the chapter on tropane alkaloids, tracing the correlation of mydriatic effects with an esterified α-hydroxyl group on C(3) (as in atropine and hyoscyamine), anæsthetic effects with esters of the stereoisomeric

3β-hydroxyl compounds (in cocaine, tropacocaine and psicaine) and central nervous system stimulation with N-substituents, irrespective of the orientation of the 3-hydroxyl group.

In contrast, the other not strictly chemical topic—that of "Alkaloids in the Plant"—has been so amply supplemented as to extend the bibliography references from 175 to 452; and to the more general reader, this could conceivably prove the most rewarding chapter in the two volumes. It is estimated that about 1,000 naturally occurring vegetable alkaloids have now been characterized, occurring in 10-20 per cent of all plants, and at all levels of the vegetable kingdom, including fungi, lichens and fern-allies. Even these figures may prove a substantial under-estimate if smaller amounts of alkaloids are looked for and recorded; and it may well be the case that there is nothing at all exceptional in the capacity to biosynthesize alkaloids, but only in the capacity to tolerate them in substantial quantities. Thus, in an example quoted, the tomato plant, though itself elaborating small amounts of nicotine, succumbs to the effect of a higher concentration when grafted on to *Nicotiana*.

By the use of radioactive tracers, considerable advances have been possible in knowledge of the site and mechanism of alkaloid biosynthesis. Ring-closure of amino-acids emerges as a widespread pattern, though not always by the most obvious sequence: while the pyrrolidine ring in nicotine, for example, is shown to originate from ornithine, radioactive lysine (the next higher homologue) did not contribute as expected to the pyridine ring, and its radioactivity appeared again in the pyrrolidine nucleus.

Work on variations of alkaloid content due to environmental factors, and to minor genetic differences between geographical races, is rather briefly summarized; and no reference is made to the protracted investigation of these factors in the opium poppy, sponsored by the United Nations to assist in tracing the currents of illicit circulation of opium.

Throughout such botanical-chemical studies, the establishment of a chemical basis for taxonomy remains as a shining objective. The wide distribution with which some alkaloids are now to be credited, however, must be a signal for caution in applying chemical criteria except within narrow and regular taxonomic groups.

Of the original 38 chemical chapters, 30 are now supplemented, affording examples both of swift expansion over new fields, and of the final conquest of ancient peaks previously reconnoitred in long and strenuous approaches. In the former category, most space is claimed by the *Rauwolfia*, *Amaryllis*, *Veratrum* and *Senecio* groups. At the date of the original volumes, knowledge of the *Rauwolfia* alkaloids covered only seven bases of unknown constitution, while from the Amaryllidaceae fifteen were known. From the same sources, the present supplement describes more than forty and more than seventy respectively, the greater number of which are elucidated as to structure, and some of which, including reserpine itself, have been synthesized. Such rapid advances have been mainly made possible by the use of chromatographic techniques for the separation of complex mixtures, and of paper chromatography and infra-red spectra as criteria of a complete separation.

In a comparable way, the use of X-ray crystallography for final confirmation of the structural formulæ, deduced from many years of degradative chemical investigation, may be said to have consolidated the jumping-off ground for the long-awaited syntheses of strychnine, morphine and hyoscine. All these are recorded in the present volumes; and surely it would be a sluggish soul that would find anything out of place when they are chronicled, with a touch of unwonted rhetoric, as a storming of citadels. E. G. KELLETT

BRITISH DEER FORESTS

The Deer Stalking Grounds of Great Britain and Ireland

Compiled by G. Kenneth Whitehead. Pp. xviii+556+38 illustrations. (London: Hollis and Carter, Ltd., 1960.) 126*s*. net.

THOUGH the subject has been tackled by three previous writers between 1839 and 1923, this is the first really comprehensive survey of deer forests in the British Isles, and certainly the first with such abundant statistics of the deer bagged. The major part of the book consists of an alphabetical list of deer forests, with the following details: acreage; area above 1,500 ft, and below 1,000 ft., highest and lowest ground; number of deer killed annually, divided into stags and hinds for both pre-1940 and post-war; average weight of stags and hinds, with weight of heaviest stag known to have been killed; and particulars of the most important heads recorded for the forest.

A series of detailed appendixes gives an alphabetical list of the owners of deer forests and shootings in 1959; a counterpart list of the deer forests with their owners; a list of properties of the Forestry Commission in or adjacent to deer-forest country; four different classifications of the best red deer heads recorded in the British Isles during the past 200 years; and a list of the heaviest wild red deer stags known in the same period. The whole is garnished with 38 excellent black-and-white photographs, a dozen useful maps showing the actual location of each forest, a bibliography and several indexes.

It is clear that the sportsman interested in deer-stalking could scarcely expect a more complete provision for his needs. For the naturalist, too, there is an immense amount of valuable material to be quarried from Col. Whitehead's research. Statistical analysis of all these figures about average weights and head sizes surely ought to produce information of much value. There is also a great deal of important information tucked away in the notes that follow the figures about each deer forest, such as when and whence deer have been introduced into them to raise the quality of the native stock, and how these introductions have fared.

Though the great majority of British deer forests are in the Highlands of Scotland, there are also a few in England and Ireland, but none in Wales. The fact that the red deer of Exmoor and the New Forest are hunted with hounds excludes them from consideration, but seven deer forests are listed for the Lake District, while introduced deer have enabled Lundy Island in the Bristol Channel, the Roches in the moorlands of Staffordshire, and Thetford Chase in the Norfolk Breckland to qualify for inclusion. In Ireland there are ten deer forests, mainly in Counties Donegal, Kerry and Wicklow.

One can have no hesitation in affirming that this very thorough piece of work will hold its own as the standard work on British deer forests until the end of the century, and probably well into the next one as well. But I commend to the Deer Group of the Mammal Society of the British Isles the task of making good use of the vast store of natural history information it contains.

R. S. R. FITTER

GEOMORPHOLOGY OF WALES

The Relief and Drainage of Wales

A Study in Geomorphological Development. By Eric H. Brown. Pp. xii+186+24 plates. (Cardiff: University of Wales Press, 1960.) 30*s*.

DURING the course of the past hundred years a considerable diversity of opinion has accumulated concerning the nature, origin and age of the erosion surfaces in Wales. Many of these views are mutually exclusive. Thus the Permo-Triassic age suggested by O. T. Jones conflicts with Jukes Browne's Cretaceous, and both with W. M. Davis's Tertiary. A. C. Ramsay's submarine hypothesis is in contradiction with W. M. Davis's subaerial argument, and so on. Equally complex is the problem of drainage evolution. T. N. George supports the view of rivers extending their courses across emergent wave-cut platforms of Pliocene age, while A. Strahan has proposed that the new discordant rivers of South Wales have been superimposed from a Cretaceous cover.

The problems of the evolution of drainage and the extent, origin and age of the erosion surfaces are closely linked; these together form the basis of the denudation chronology for any given area. For the first time for Wales these two lines of inquiry are brought together in Dr. Eric Brown's study of the relief and drainage of the whole principality. The survey is thus in the tradition of the classic accounts of the French *Plateau Central*, by Henri Baulig, and *Structure, Surface and Drainage in South East England*, by Profs. Wooldridge and Linton.

This new book is the product of many years of study and much field work, and gives for the first time a systematic geomorphological account of much of the landscape of Wales. Although some of the conclusions advanced by Dr. Brown are open to discussion, all those interested will applaud his introductory survey of the relief and structure of Wales and his masterly account of the views put forward during the past hundred years to account for these features.

In the second half of the book Dr. Brown advances his own interpretation of the denudation chronology in considerable detail. Evidence is submitted for the existence of at least three high-level subaerial peneplains of early Tertiary age above 700 ft.: and below this height for a series of coastal plateaux, notably at 600-, 400-, 300- and 200-ft. levels, of submarine origin and Pleistocene age. The drainage evolution is worked out in relation to these erosion surfaces. Forty-seven maps and diagrams and twenty-four plates support the text, and the account concludes with a comprehensive bibliography.

The bibliography will be of great value to future research workers in this field, but it is perhaps unfortunate that there is no reference to Dr. Brown's paper on "The Physique of Wales" given to the Royal Geographical Society on October 29, 1956 (*Geographical Journal*, 123, Part 2, June 1957). This paper is of great interest and value, as the nature and extent of the problems still outstanding after Dr. Brown's analysis and synthesis of the geomorphology of Wales clearly emerge in the discussion which followed. One other addition to the book might have been considered—a short glossary of Welsh landscape terms. So many Welsh place names arise from the topography that additional interest would doubtless be added for the English reader if an indication of the meanings were made available.

W. G. V. BALCHIN

INTRODUCING METEORITICS

Principles of Meteoritics

By E. L. Krinov. Translated from the Russian by Irene Vidziunas. Translation edited by Harrison Brown. (International Series of Monographs on Earth Sciences, Vol. 7.) Pp. xi+535+7 plates. (London and New York: Pergamon Press, 1960.) 70*s*. net.

THE original edition of Krinov's introductory book on the study of meteorites has been available in Western meteorite laboratories for some years, but it is doubtful whether many investigators have actually read more than the sections of particular interest to their own work. It is essentially an introductory text, and, as such, may be read with profit by the sort of informed lay public at which Sir Lazarus Fletcher's British Museum guide was directed; but there is also included a section on the external appearance of fusion crusts which represents an important original contribution to the subject by the author himself. D. R. Chapman's recent article on tektites (*Nature*, 188, 353; 1960) indicates how profitably such work may be extended by aerodynamicists.

The treatment is naturally from a Russian point of view, and most of the specialist articles quoted are of Russian origin, but a real effort was made in the original edition to acknowledge the importance of work by Prior and Hey, Perry, Urey and others. The translated version also includes a selection of both Russian and American work published since the original edition.

In so far as I am able to check it, the translation appears very faithful. In the original, the text and legend are at one in their misinterpretation of Fig. 121, whereas in the translation the text follows the original, but the legend (Fig. 129 of the translation) has been altered to a factually correct form.

Several additional illustrations have been incorporated in the translated edition, and some of the original illustrations have been replaced by more up-to-date or clearer ones. In a work of this nature the half-tone illustrations are of great importance, and it is very unfortunate that in reproduction some of them have deteriorated. On the other hand most, but not all, of the new illustrations are of good quality. A very serious loss in the translated edition is the totally inadequate version of Fig. 52, but this could easily be corrected.

H. J. AXON

Vistas in Free-Radical Chemistry

Edited by Dr. W. A. Waters. (International Series of Monographs on Organic Chemistry, Vol. 1. Supplement No. 3 to *Tetrahedron*). Pp. ix+251. (London and New York: Pergamon Press, 1959.) 75*s*. net.

THIS book is published in memory of Dr. Morris S. Kharasch, and is a fitting tribute to one of the great organic chemists of this century. A complete list of the published papers of Kharasch and his collaborators is given, and is followed by a selection of twelve papers which are presented in full; these include descriptions of Kharasch's early work on the discovery and exploitation of the 'peroxide effect' and some recent work on polymerization reactions. Many of us have a lesson to learn from Kharasch's attention to detail, which has led to several important discoveries. F. R. Mayo and C. Walling pay personal tributes to Kharasch, and give very interesting accounts of their work in collaboration with him. An account of modern work and trends in free-radical reactions in solution is given by W. A. Waters.

The book concludes with chapters on homolytic aliphatic and homolytic aromatic substitution, by H. C. Brown and D. H. Hey, and some other aspects of free-radical chemistry, including a concise account of free-radical reactions in gases by K. O. Kutschke and E. W. R. Steacie. The object of including these chapters is to illustrate the effect of Kharasch's research on the development of free-radical chemistry; this is now such a large subject that a comprehensive treatment would not be possible in only 250 pages. The aspects selected will be, on the whole, of interest to organic chemists, particularly those working on free-radical reactions.

The volume is a useful historic record of Kharasch's contribution to free-radical reactions in solution.

A. B. Callear

Public Relations and Management

By David Finn. (Reinhold Management Science Series.) Pp. xiii+175. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1960.) 36*s*. net.

SUSPICION of those who court publicity has led the British people to look askance at men and women who act as public relations officers for their organizations. This suspicion has been strengthened by some of the public relations practitioners whose efforts to influence the Press have been, to say the least, somewhat unsavoury.

Against this canvas, genuine public relations officers have had difficulty in establishing themselves and, particularly, in showing that their jobs consist of considerably more than preparing hand-outs for the Press. Yet many companies are becoming aware that it is not enough to provide needed goods and services for the community; to obtain maximum results the company must present an image to the public which will persuade more and more people to make use of those goods and services. The public relations officer is concerned to influence public opinion favourably towards his company.

David Finn is an experienced public relations consultant in the United States and, in this book, does much to show the worth of effective public relations to a company. He strips the gloss from the brassy publicity-seeking public relations officer, and illustrates how the practitioner can create a favourable climate of opinion for his company by examining every corporate act to see what effect it will have on public opinion and how projects should be designed to win public support. Besides managing directors, this modest, well-reasoned case should appeal to many heads of institutions outside the world of industry.

T. H. Hawkins

Homotopy Theory

By Sze-Tsen Hu. (Pure and Applied Mathematics, Vol. 8.) Pp. xiii+347. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1959.) 11 dollars.

A COMPREHENSIVE introductory account of the rapidly growing field of homotopy theory is welcome; the present volume is rather less concise than Hilton's Cambridge Tract, and differs from it somewhat in content. It is not altogether easy reading, and a really sound knowledge of elementary algebraic topology is an essential preliminary. The main problem in homotopy theory is the extension problem studied in Chapters 1 and 2; fibre spaces and mapping spaces fill Chapter 3. The next four chapters deal mainly with homotopy groups and cohomotopy groups, and the final group of four chapters is concerned chiefly with spectral homology theory. Specialists in a domain which is expanding so quickly may quarrel with some of the omissions; but the young topologist must be grateful for so well-balanced and clear an account. There are exercises to each chapter, but the reader must be warned that these range from the trivial to the impossible, the latter being those which merely summarize results to be found in the periodical literature of the subject; a clear separation between genuine exercises and bibliographical annotations might have been a better plan.

T. A. A. Broadbent

Dictionary of Nutrition and Food Technology

By Dr. Arnold E. Bender. Pp. vii+143. (London: Butterworths Scientific Publications, 1960.) 30*s*.

DR. BENDER'S *Dictionary* sets out to interpret the technical terms used by the many different scientific and practical groups who take part in the expanding knowledge of nutrition. Practical workers in food technology may well be lost in the jargon of the biochemist, while the scientific workers may need definition of technological terms. The dictionary covers the most common biochemical, bacteriological, chemical and technological processes in food science, with brief descriptions also of equipment used in the laboratory and in manufacturing processes.

The sources of information listed in the introduction to the dictionary provide a useful nutrition bibliography, although there are some serious omissions, such as the failure to give full references for the publications of the U.S. National Research Council and the Nutrition Committee of the British Medical Association. Some of the information on special diets is not from the most up-to-date sources and some misleading statements, such as the correlation of glossitis with ariboflavinosis, need to be corrected. The choice of foods for which composition figures are given seems rather arbitrary, and the sources of the food analyses need some further explanation. However, in spite of such flaws, the dictionary is a useful addition to the nutrition bookshelf and should solve many questions of terminology.

A. M. Copping

AUSTRALIAN UNIVERSITIES

By Sir ERIC ASHBY

Clare College, Cambridge ; formerly Professor of Botany, University of Sydney

IN 1957, a Committee appointed by the Prime Minister of Australia, under the chairmanship of Sir Keith Murray (chairman of the University Grants Committee in Great Britain), issued a report which has brightened the prospect for Australian universities. One outcome of the Murray Report was the creation of an Australian Universities Commission, which consists of five members : a full-time chairman (Sir Leslie Martin, formerly professor of physics in the University of Melbourne), two members drawn from academic life, and two from industry.

The Commission has presented its first Report*. This summarizes the development of the universities over the triennium 1958–60 in student-numbers, staff and finance. It then goes on to set out the Commission's recommendations for the triennium 1961–63 ; and it has chapters on halls of residence and on the future of Australian universities.

It is a record of remarkably rapid expansion since the date of the Murray Report, and a prediction of further rapid expansion over the next three years. The following figures (selected from tables in the Report) illustrate the rate of growth of Australian universities :

	1950	1952	1960
Enrolments	—	27,325	53,478
Academic staff (State universities)	—	1,645	2,972
Recurrent income (£1,000)	3,756	—	22.677

The figures for enrolments indicate that nearly 6 per cent of the 17–22-year-old age group are enrolled at universities ; but the figures have to be interpreted with caution, for only 32,349 of the enrolled students (60·5 per cent) are attending universities full time. No fewer than 15,997 students (29·9 per cent) are taking part-time courses and 5,132 students (9·6 per cent) are external. The figure, therefore, which is comparable with those published by the British University Grants Committee is 32,349 full-time students in 1960. Even so, Australia already has a somewhat higher proportion of her young people at universities than Great Britain has.

The figures for numbers of academic staff also have to be interpreted with caution, for it is characteristic of State universities in Australia that great use is made of part-time staff. However, when the figures for enrolments and for academic staff are adjusted to take account of the fact that there are part-timers in both categories, the student-staff ratio comes out at about 14 : 1, compared with an average for British universities of about 9 : 1, and for American universities, colleges and junior colleges of 13 : 1.

The figures for recurrent income reflect first of all Commonwealth participation in university finance (an increase from £0·388 million in 1950 to £7·610 million in 1960), together with substantial increases in grants from State Governments (from £1·616 million in 1950 to £8·727 million in 1960) and in student fees (from £0·697 million in 1950 to £3·039 million in 1960). It is noteworthy that this fee-income is about the same as the income from fees from 63,068 full-time students in eighteen English universities and colleges in 1954–55.

Notwithstanding the recent rapid expansion of Australian universities, the Commission sees no alternative ahead but further rapid expansion. The Report predicts that by 1966 there will be at least 95,000 students enrolled in Australia's ten universities. It is estimated that the University of Sydney alone will have 16,300 students and the University of Queensland 18,000.

The Commission does not disguise the fact that this immense increase in enrolments will precipitate problems both of standards and of staff. Already such data as are available indicate that fewer than half the students at Australian universities graduate in the minimum time. The Commission does not recommend a stiffening of the standards for entrance to universities, nor an increase in maturity of freshmen (which could be secured by raising the age of entry to universities). So as numbers increase the prospect is that student-performance will deteriorate rather than improve.

This possible deterioration might be corrected by a very substantial improvement of the student-staff ratio (though there is no convincing evidence to support this belief). The Commission estimates that 3,613 university teachers, over and above the present numbers (an increase of more than 100 per cent), will be needed by 1966. To recruit academic staff in these numbers is the most perplexing problem facing the universities of Australia.

Comparisons of costs between British and Australian universities are very difficult to make, and the figures given in the Report (£A.448 per student in Australian State universities compared with £A.562 per student in eight 'representative' British universities) should not be compared without qualifications. The case, however, for increased grants to Australian universities needs no such comparison to support it. After surveying the needs of the universities in detail the Commission makes the following recommendations for the triennium 1961–63. The figures are in millions of pounds (Australian).

	Commonwealth share	State share
State universities		
Recurrent (spread over 1961–63)	25·424	47 040
Capital (including building, furnishings and equipment, 1961–63)	15 385	15 385
Total (1961–63)	40 809	62·425
Australian National University		
Recurrent (spread over 1961–63)	9·250	—
Capital (including building, furnishings and equipment, 1961–63)	5 156	—
Total (1961–63)	14 406	—

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* Report of the Australian Universities Commission on Australian Universities 1958–1963. Pp. vi+77. (Commonwealth Government Printer, Canberra, 1960.)

If these recommendations are accepted the overall expenditure on universities from Government sources (both State and Commonwealth) in the triennium 1961–63 will be nearly £118 million.

In commenting on the prospect which confronts higher education in Australia, the Commission encourages universities to explore fresh ways to make the best use of their available accommodation and high-level man-power. It mentions a submission received from Prof. H. C. Webster in Queensland, outlining a revision of the university calendar which would lead to doubling of utilization of lecture rooms and laboratories, and an experiment already operating in the University of Pittsburgh which allows some students to spend eleven months instead of nine each year on formal academic work. The Commission also suggests that some consideration might be given to the creation of 'satellite' colleges, associated with parent universities, for first-year students. Australia already has experience of this, for the University of Melbourne set up a temporary first-year college near Mildura at the end of the Second World War. It is clear from a reading of the Commission's Report that the Australian universities, which in their constitution and administration are modelled on Scottish universities, have reached a critical stage of their history. Their size has already outgrown their structure. The Commission is evidently giving very careful thought to their future, and is encouraging the universities to propose solutions to the problems which confront them.

PSYCHOLOGY AND THE INDIVIDUAL

By G. W. H. LEYTHAM
Department of Psychology, University of Liverpool

MORE than a hundred years ago, John Stuart Mill differentiated between Psychology, the science of mind, and Ethology, the science of character: "The one ascertains the simple laws of Mind in general, the other traces their operation in complex combinations of circumstances. Ethology stands to Psychology in a relation very similar to that in which the various branches of natural philosophy stand to mechanics. . . . Ethology, the deductive science, is a system of corrolaries from Psychology, the experimental science". ("A System of Logic", 1843.)

The development of psychology has been fraught with dichotomies of this kind, but their proponents have usually been much more partisan than was Mill, and they have been more inclined to regard the two views as being in conflict rather than as complementary. While the academic Wundt was founding the first psychological laboratory at Leipzig in 1879, for the study of the generalized, adult, human mind, Charcot, the medical therapist, was busy in Paris studying the effects of hypnosis on mental patients. The former led to experimental psychology and schools, such as Structuralism, Gestalt and Behaviourism, while the latter paved the way for Freud's psychoanalysis and the divergent theories of Jung and Adler.

Already, in 1894, the German philosopher Dilthey was objecting to the narrowness of experimental psychology, with its explanation and analysis (*Erklärende Psychologie*) and its use of the techniques of natural science (*Naturwissenschaft*). He wanted psychology to study character or human values and to be concerned with understanding and description (*Verstehende Psychologie*). The individual was to occupy a central place in this approach, and psychology would thus serve as a basis for the cultural and social sciences (*Geisteswissenschaft*). In the same year, another philosopher, Windelband, characterized the two approaches as the 'nomothetic' and 'idiographic' respectively. He also felt that the latter was truly scientific, and should be the method followed by psychology.

This distinction between the search for general laws and a concern for the uniqueness of the individual has since shown itself in the gulf which has at times separated the experimentalist from the clinician, the psychologist from the psychiatrist, and, in a wider sense, the pure or theoretical scientist from the applied or practical scientist. It can even be seen in the gap between the sciences and the arts, but in none of these is it necessary or desirable: the aims of the two approaches are different but not antagonistic. Mill said that: "Art in general, consists of the truths of Science, arranged in the most convenient order for practice, instead of the order which is most convenient for thought," and according to his definition, ethology was "the science which corresponds to the art of education; in the widest sense of the term, including the formation of national character as well as individual".

In his presidential address to the British Psychological Society in 1958, Rodger spoke about "Psychologists: Non-medical and Medical"[1], and discerned at least three dichotomies in the history of psychology: cognitive versus non-cognitive (what is studied); experimental versus non-experimental (how it is studied); and laboratory versus extra-laboratory (where it is studied). He pointed out that those working in one 'cube', for example, carrying out experiments in the laboratory on cognitive processes, should realize that theirs was only one room in a large house, and that they should not ignore or belittle the work going on in the other and more distant ones, for example, observations of neurotic behaviour made in the clinic.

The conciliatory nature of Rodger's address may well be taken as a sign of the times, for there are encouraging indications that as psychology emerges from its reviewing phase, it is inclining away from the old theses and antitheses towards a new synthesis. Take, for example, the series of papers on modern trends in psychology[2] read at the annual conference of the British Psychological Society in 1960. The first was concerned with "perception and information theory" and indicated some of the ways in which the mathematical theory of communication deriving from Shannon and Weaver (1949) could be applied to perceptual data. In this approach, the limitations of which are noted, the stimuli are regarded as 'input', the subject as a 'communication channel', and responses as 'output'. The paper on behaviour theory pointed out that the large-scale, controversial theories, such as those of Tolman and Hull, were going out of fashion and were being replaced by an emphasis on fact finding and the construction of mathematical models based on a probabilistic conception of behavioural processes. Models using concepts from electronics or communication engineering, and having an obvious connexion with cybernetics (Wiener, 1947), were also being used. The contribution on social psychology welcomed the rapidly diminishing use of stimulus-response language in that field, and noted an increased stress on human interaction and language. One systematic approach to the former owed much to the theory of games, while the latter could be studied in terms of information theory, or as an important aspect of the sorts of constructs a person builds up to make his world more predictable, and to use in identifying and evaluating his social environment.

In the remaining paper, on thinking, Allport is quoted as saying that our entire mental life is dominated by a process whereby experiences are formed into clusters, concepts, or categories. While this interest in concept formation and language obviously relates the psychology of thinking to social psychology, one can also see affinities with the other two fields already mentioned: current research in thinking is largely exploratory, and some of it stems from analogies drawn between thinking and information-processing computers. The use of probabilistic inference in the solving of syllogisms has also been investigated. The kind of strategy a person will use to solve a problem seems to depend largely on his previous experience; hence there is also a connexion with personality studies and with a consideration of the individual.

Perhaps this fresh impetus in the long-neglected study of human thinking and language will hasten the reconciliation of the nomothetic and idiographic approaches and bring us nearer to the day when the truly complementary nature of Mill's two sciences will be realized. This would also seem to be the opinion of Hearnshaw who, in his presidential address to the British Psychological Society in 1956[3], spoke of the tension "between the demands of scientific method and the appreciation of the richness of human individuality". He said: "I can see only one way of narrowing the gap between the scientific and humanistic psychologies, and that is through a gradual encroachment of scientific techniques upon territory already roughly mapped by humanistic insight. This means in effect a constant search for concepts which, while capable of scientific definition and employment, nevertheless possess humanistic implications in the sense that they throw light upon peculiar, or, as they are sometimes termed, the higher attributes of men". His own interest in the psychology of thinking has centred around temporal integration, which he regards as one such bridging concept.

Allport, in his book "Becoming" (1955), considered that: "Virtually all modern psychological theories seem oriented toward one of two polar conceptions . . . the Lockean and the Leibnitzian traditions respectively". Whether this be regarded as a dichotomy or a continuum, it can be seen to be very much in line with the scientific–humanistic conflict inherent in the other pairs of opposites. At the Lockean end, man is a *tabula rasa* reacting to external forces, while at the Leibnitzian end, he is the monadic source of all his behaviour. Put in these extreme forms, the inadequacy of either taken by itself becomes obvious, and one can see that although some theories in psychology are presented as if they accounted for the whole of man's behaviour, they do in fact only refer to part of it. Many theories are thus mutually exclusive rather than contradictory, and complementary rather than in conflict. Allport felt, however, that psychologists had tended to concentrate too much on the lower end of the scale, and he called for further scientific studies of the later and higher levels of development, including growth, adulthood, and man's religious sentiment. Such investigations would serve to augment many already carried out on rats, children and neurotics, and as he saw it: "The major task of psychology to-day is to enlarge its horizons without sacrificing its gains".

Several straws in the wind suggest that Allport's dictum is being taken seriously, for apart from the renewed interest in the psychology of thinking mentioned above, the study of mature people made by Maslow, and reported in his book, "Motivation and Personality" (1954), has opened up a new field of inquiry, and the numbers interested in psychological health and the potential greatness of man has grown so rapidly that a new periodical, *The Journal of Humanistic Psychology*, is to start publication very shortly. The new American *Journal of Existential Psychiatry* also provides a forum for those who wish to "help shape the picture of an organism at once mechanical and random, statistical and individual, limited and free", and is evidence of the increasing rapprochment between the humanized behaviourism of the United States and the Continental stress on man-in-action and phenomenology.

The recent "Handbook of Aging and the Individual" (1959), edited by J. E. Birren, reveals a growing interest in adulthood and old age, and the study of human phenomena not at the moment susceptible to high levels of numerical description has been greatly facilitated by the appearance of Siegel's "Non-parametric Statistics for the Behavioral Sciences" (1956).

A pioneering study by Harlow on "The Nature of Love"[4] has shown how problems of the clinic and everyday life can be investigated experimentally in the laboratory, and serves to emphasize that techniques are of secondary importance. Provided he asks worthwhile questions, the psychologist has no need to apologize because his laboratory does not resemble a real-life situation. A vacuum is scarcely representative of the physical world, yet Lord Kelvin once said that we knew more about a vacuum than we knew about anything else. As, in going from a vacuum to the everyday physical world, allowance must be made for the properties of the particular object and the forces acting on it, so, in going from the laboratory to the everyday psychological world, we must allow for the heredity and experiences of the particular individual and the psychological forces present in his environment. Presumably this is what Mill meant when he said that psychology was the science of the elementary laws of the mind and ethology "the subordinate science which determines the kind of character produced, in conformity to those general laws, by any set of circumstances, physical and moral".

Einstein said: "The object of all science, whether natural science or psychology, is to coordinate our experiences and to bring them into a logical system". ("The Meaning of Relativity", p. 1.) The experiences of psychologists have been so diverse that one would expect their logical systems to be varied too. Observations of the behaviour of rats running in mazes may well lead to a theory quite different from one based on listening to the free-associations of neurotics lying on couches. These are complementary theories, however, and not alternatives: each is limited and applies to a different situation.

The big need in psychology to-day is for a conceptual framework in which all the experiences and theories can be related to each other. We have theories for many of the bits, but so far there is no satisfactory theory of the whole. Such a theory would not only have to bring together and relate the various aspects of behaviour, but would also have to take account of the fact that development and growth are psychological as well as biological.

As a first step in this direction, it would be helpful to have an agreed unit for the science of psychology. The most obvious and useful one is that of the individual, but although lip-service is often paid to this it is frequently reduced to a physiological organism or an inanimate machine, and is rarely described in psychological terms derived from a consideration of the integral and extensive nature of its behaviour. In an attempt to establish the individual as the unit of psychology, I would like to introduce a model of it which I have been developing over the past few years, and which I have found helpful as an integrating framework.

A diagram of the structure of the individual is given in Fig. 1, and although a detailed description of its dynamics is not possible here, it is hoped that the following summary will give some idea of its possibilities in expediting the above-mentioned trends towards the extension and unification of psychology.

Introductory text-books in psychology seem to have grown by accretion rather than by design, and there is a certain autonomy about their chapters which makes it difficult to appreciate the essential unity of the individual's behaviour. How would the proposed model cover the topics of the various chapters and help to bring them together into a unified whole?

The social, physical and biological environments impinge on the individual at the nucleus, which is both a transformer and a relay station: it transforms incoming stimuli and relays them to the ego and also receives outgoing stimuli from the ego and transforms them into action or responses. Psychologists studying the relationship between the behaviour of the psychological individual and the activities of the biological organism, or with the machine-like aspects of behaviour, would be concerned mainly with the properties and functions of the nucleus. Those interested in problems of sensation would find the physical level of awareness and its relation to the nucleus relevant to their work. Feeling and emotion would, of course, have special reference to the emotional level of awareness, while reasoning and other activities involving the use of language would come within the sphere of the mental level of awareness. Certain aspects of creative thinking and the higher forms of religious experience would be referred to the intuitive level of awareness.

The ego can operate on any level of awareness and represents immediate experience or the perceptual world, from which the social, physical and biological worlds are inferred. Problems of perception would thus relate to the dynamics of the ego and the particular level of awareness on which it was functioning. Learning would also apply to all levels, and it seems likely that psychological time (the 'specious present') would correspond with increasing spans of physical time at successive levels of awareness away from the nucleus.

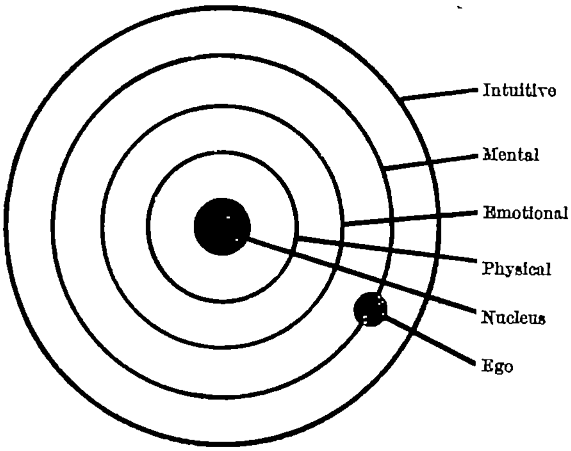

Fig. 1 A model of the individual, showing the four levels of awareness, the fixed nucleus, and the mobile ego

The successive levels of awareness are also related to the successive stages in psychological development, and these may be understood with reference to the range and preferred focus of ego functioning. The model accords well with Maslow's theory of motivation, which states that "the basic human needs are organized into a hierarchy of relative prepotency". As a 'lower' need becomes satisfied, or assured of satisfaction, so the next 'higher' need emerges and becomes central in guiding behaviour. Maslow's hierarchy of needs, together with the aspect of the model of the individual to which they seem relevant, are as follows: physiological needs (nucleus); safety needs (physical level of awareness); belongingness and love needs (emotional level of awareness); the esteem needs (mental level of awareness); and the need for self-actualization (intuitive level of awareness).

It will be seen that Jung's four functions—sensation, feeling, thinking and intuition—also bear a close resemblance to the four levels of awareness. So far as possible, static terms such as 'the unconscious' would be avoided in this model and would be replaced by dynamic relationships, for example, relative availability to the ego as determined by comparative thresholds of arousal.

As we come to know more about the unity of the individual and the way in which its different parts are integrated to function harmoniously, so may we come to understand not only optimal psychological growth, but also disintegration and disharmony. A useful model would thus help us to ask the right questions and guide our search for fresh facts, as well as enhancing the value of the facts already known by bringing them together and relating them in a dynamic system.

How would the suggested model help to bridge the gap between the pure and applied fields of psychology? The structure and dynamics of the abstract, theoretical individual, would be the concern of the pure scientists, while the application of the generalities thus found to the life and experiences of the particular individual in his particular situation would constitute the aims of the applied psychologists. Knowledge obtained in this latter field would be 'fed back' to the theorists and experimentalists to help them in the modification of their model and in the formulation of vital problems for research. The two aspects of psychology would thus be complementary and mutually helpful, and the answer to the question implied in the title of Lafitte's book, "The Person in Psychology: Reality or Abstraction" (1957), would be 'both': reality in applied psychology, and abstraction in pure or theoretical psychology.

I have tried to show that the time now seems ripe for an attempt to integrate and unify the many facets of psychology, and have proposed a model of the individual as a contribution towards this. Should it prove helpful to psychologists as a logical system whereby they can co-ordinate their experiences, then the similarity between its structure and those of the atom and the solar system may also take us a step nearer to that more distant goal of the unification of all science.

[1] Rodger, A., *Bull. Brit. Psychol. Soc.*, No. 36, 1 (1958).

[2] *Bull. Brit. Psychol. Soc.*, No. 42, 23 (1960).

[3] Hearnshaw, L. S., *Bull. Brit. Psychol. Soc.*, No. 30, 1 (1956).

[4] Harlow, H. F., *Amer. Psychologist*, 13, 673 (1958).

THE COSMIC RAY FLARE ON NOVEMBER 12, 1960, AND SOLAR ACTIVITY DURING THE PERIOD NOVEMBER 10–15, 1960

By STAFF OF THE IONOSPHERE AND RADIO-ASTRONOMY SECTION OF THE NETHERLANDS POSTAL AND TELECOMMUNICATIONS SERVICES

THE region of solar activity which passed the central meridian on November 12, 1960, at heliographic latitude 27° N. produced a remarkable series of flares, two of which caused cosmic-ray effects at ground-level. In this communication we give a short account of the optical, radio-astronomical, cosmic-ray and earth-current observations made at the Nera Observatory of the Netherlands Postal and Telecommunications Services and at the observing stations at Paramaribo (Surinam) and Hollandia (New Guinea).

The principal flare events belonging to this series are summarized in Table 1.

The radio responses associated with these flares were all spectral type IV, consisting of prolonged continuum radiation on both centimetre and metre wave-lengths. The durations listed for 200 Mc./s. in Table 1 apply to the main part of the events only. After the flare of November 10, radio noise at this frequency continued to be enhanced up to the early hours of November 13. Initially this enhanced radiation showed relatively little variability, but on November 12 strong activity of type I storm-bursts developed. The flares of November 14 and 15, on the other hand, did not provoke prolonged enhanced or storm radiation.

The flare of November 12 is comparable in importance to that of the famous cosmic ray flare of February 23, 1956. Some of the features of this event will be described here.

Optical Development

Fig. 1 shows some photographs in H_α light of this flare taken at Nera. Notable features of this phenomenon are the following: (1) the undulatory structure of the north-eastern branch (the upper branch in Fig. 1) which was especially pronounced at about 1328 U.T.; (2) the very bright portions between the two big spots and the conspicuous bright knot on the northern side; (3) the very rapid extension of the flare in the south-westerly direction between 13h. 27·5m. and 13h. 28·5m.; (4) the coverage of the nearby spots by luminous material around 1334 U.T.

Table 1. IMPORTANT FLARE EVENTS BELONGING TO THE ACTIVITY REGION WITH CMP ON NOVEMBER 12, 1960
(9,400 Mc./s. data taken from Japanese Ursigram information)

Date	Optical flare		Radio response				Geomagnetic activity
	Position	Importance	Frequency (Mc./s.)	Start time (U.T.)	Duration (min.)	Integrated flux density (10^{-19} × W.m^{-2} (c./s.)$^{-1}$ min.)	
November 10	28° E 29° N.	3	2,980 545 200	1119 1116 1116	33 28 44	1,200 4 500	
„ 11	— —	—	9,400 545 200	0311 0318 0321	104 160 250	— 320 700	
„ 12	2° W. 27° N.	3 + (C.R.)	2,980 545 200	1322 1327 1327	100 100 270	34,000 100? 80	SSC 1348 U.T. (storm : 1710 →)
„ 13							„ 1022
„ 14	20° W. 28° N.	2 +	9,400 545 200	0330 0335 0319	— 120 140	— 12 15	
„ 15	32° W. 30° N.	3 + (C.R.)	9,400 545 200	0217 0221 0221	41 160 238	— 10 40	SSC 1303 storm 2202 →

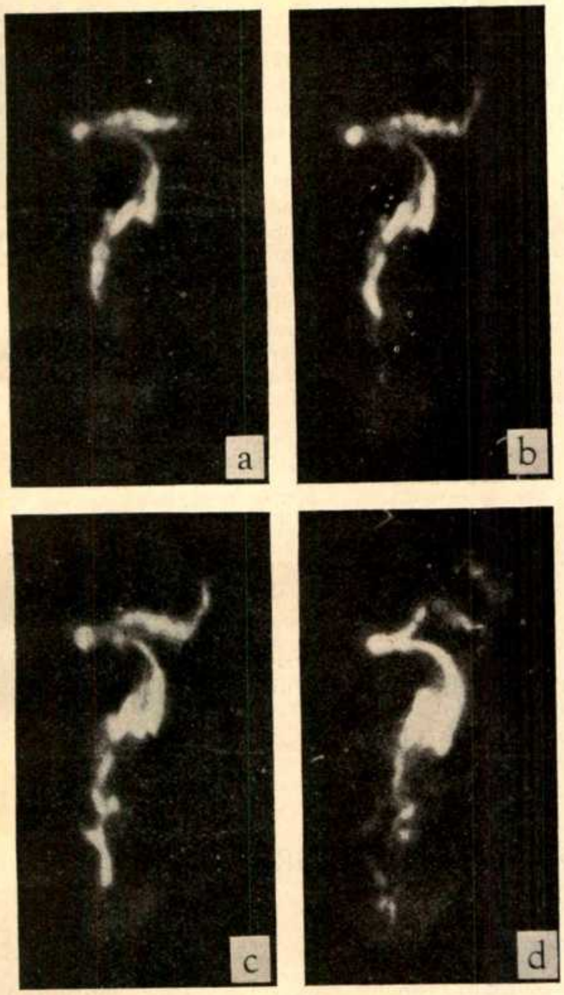

Fig. 1. Photograph in H_α radiation of the flare of November 12. (*a*) 13h. 27m. U.T.; (*b*) 13h. 27·5m. U.T.; (*c*) 13h. 28·5m. U.T.; (*d*) 13h. 34m. U.T.

The overall structure of this flare was very much the same as the structure of a flare earlier in the day, observed at 1012 U.T. at the same position. These two flares illustrate nicely the well-known fact that flares within the same region of activity tend to recur along similar structural patterns.

Radio Responses

A small initial rise at frequencies 9,100 and 2,980 Mc./s. began at 1322 U.T. The onset of the main steep rise occurred at 1324. At frequencies 545 and 200 Mc./s. the onset occurred definitely later, at 1326·5 and 1327·5, respectively. The intensity curve of the unusually strong outburst at 2,980 Mc./s. is reproduced in Fig. 2.

The polarization behaviour at 200 Mc./s. was as follows. During the first 3·5 min., from 1327·5 to 1331, the Nera polarimeter indicated very irregular polarization, fluctuating between right-handed and left-handed. The degree of polarization is unknown, because the total radiation recorder was at first largely off-scale; but it was certainly very small. The irregular polarization behaviour suddenly came to an end at 1331. Perhaps this marks the end of a 'first part' event, which may have been a type II outburst. From 1331 on, the radiation showed about 35 per cent uniformly right-hand polarization. This sense of polarization, which was prevalent throughout the period under discussion, is opposite to the left-handed sense normally associated with sources on the northern hemisphere during the present sunspot cycle.

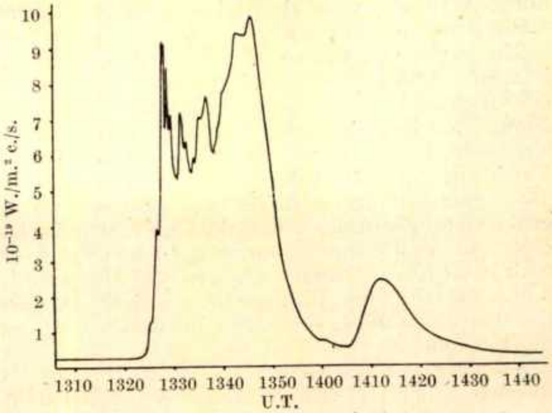

Fig. 2. The outburst at 2,980 Mc./s. on November 12

Cosmic Rays

Observations of the low-energy part of the cosmic radiation are obtained at Nera with a standard duplex neutron monitor. At about 1400 U.T. on November 12 the intensity increased, to reach a first maximum of 13 per cent above the initial level at about 1500. A second increase started at about 1745, to reach a maximum of 56 per cent at about 1930 (Fig. 3). This rise was approximately coincident with a strong increase of geomagnetic activity at

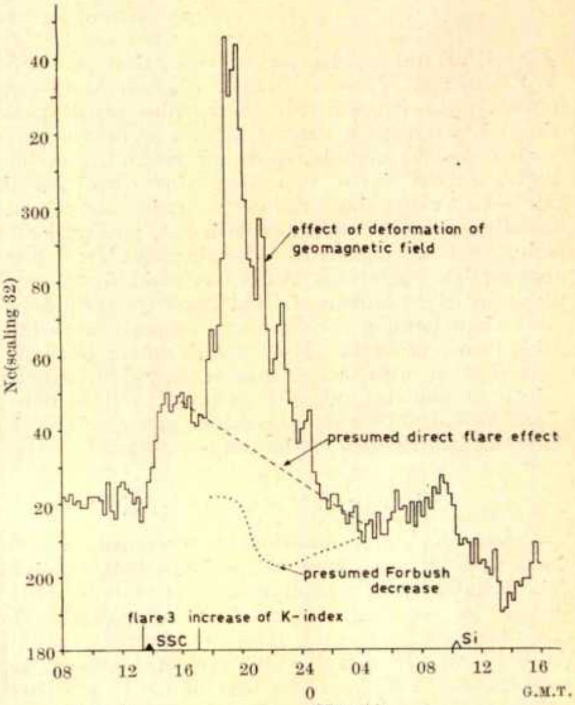

Fig. 3. Cosmic-ray flare effect observed with standard duplex neutron monitor at Nera. The diagram represents 15-min. values, corrected for air pressure

about 1710, following the sudden storm commencement (SSC) at 1348.

The increase of cosmic-ray intensity at about 1745 is most probably due to a lowering of the Störmer cut-off energy valid for the geomagnetic latitude of Nera (53·9°). This lowering of cut-off energy is obviously a consequence of the deformation of the Earth's magnetic field during the violent stage of the geomagnetic storm. Increases in cosmic rays at geomagnetic latitudes lower than normal have been recorded several times during geomagnetic storms with balloon equipment[1]. The event of November 12 represents the first instance in which it has been possible to establish this effect by means of ground observations.

At lower latitudes a Forbush decrease was observed towards 22h. U.T. This decrease is masked in our observations by the cosmic-ray flare effect, except perhaps for the later stage of its recovery (see Fig. 3).

The cosmic-ray increase on November 15 amounted to 15 per cent. The effect had a very regular course and lasted 10 hr.

Earth Current Observations

The cosmic-ray flares of November 12 and 15 were also distinctive in producing exceptionally large solar-flare effects ('crochets') in earth potential measurements at the equatorial stations Paramaribo and Hollandia, respectively.

In the period under discussion, there were two sudden storm commencements. In Table 1 we indicate the flares that can be held responsible for these SSC's. The two flares of November 12 and 15 did not cause an SSC proper, probably because their contribution to geomagnetic activity was superimposed upon already existing disturbed conditions. However, their influence can be traced tentatively in the following way: (1) on November 13 at 1022, 21 hr. after the November 12 flare, a sudden impulse of world-wide character occurred, followed by strongly reinforced geomagnetic activity lasting for several hours; (2) on November 15 at 2202, almost 20 hr. after the November 15 flare, there occurred a world-wide reinforcement of geomagnetic activity, lasting for several hours.

We note the remarkable parallelism in the sequences of events which results from these two suggested connexions:

Flare	Geomagnetic event	Interval (hr.)
November 11, 0311	SSC, November 12, 1348	34·6
November 12, 1322	*s.i.* + increased storminess, November 13, 1022	21·0
November 14, 0336	SSC, November 15, 1303	33·5
November 15, 0217	Increase of storminess, November 15, 2202	19·8

These two sequences were separated by a period of relative quietness setting in on November 14 at about 0200 U.T.

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[1] Winckler, J. R., *J. Geophys. Res.*, **65**, 1331 (1960).

THE EYE, THE BRAIN AND LAND'S TWO-COLOUR PROJECTIONS

By Dr. W. A. H. RUSHTON, F.R.S.
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GREAT interest has arisen from the spectacular demonstrations of Land[1] wherein the superposed projections of two black-white pictures, one through a red glass, can result in a scene filled with varied colours, including good greens and even some blues. Many have said that this disproves the 'classical' view that vision is trichromatic, since Land's colours are obtained with only two projection lights and are named as belonging to almost every region of the spectrum. A few have said (for example, the scholarly comment of Judd[2]) that the results show little that should surprise anyone properly acquainted with previous work. However this may be, it is a fact that in ordinary circumstances red mixed with white is called 'pink' (desaturated red); but in Land's conditions it may be called 'green'. What has happened to the eye or the brain?

Additivity in Monocular Matches

Colour-naming is notoriously unquantitative and hard to analyse, so here we rely on colour matching. The Land projection equipment was built by Dr. A. Karp[3] in the Cambridge University Engineering Laboratories, and lent to me by courtesy of Prof. Hawthorne. Red and white projection lights were superposed, each variable in intensity by partly crossed 'Polaroids'. The picture was a square subdivided into 25 small squares each uniform but differing in the red/white ratio from its neighbours in haphazard fashion. Each small square subtended 1° at the eye and the appearance gave a patchwork in many colours from red to bluish.

Interposed between the observer and the picture was a small white card about one-third the area of a small square. It did not receive any light from the projectors, but was illuminated by a variable mixture of monochromatic lights, red (650 mμ), green (530 mμ) and blue (460 mμ), from a Stiles double monochromator[4], the energies of each component R, G, B being measured by interposing an E.M.I. photomultiplier tube.

Experiment 1. By suitably adjusting the RGB mixture, the card could be made indistinguishable from the small square on which it appeared to lie; for example, seen against a green square the card could be made an identical green. The red projector light was then extinguished, so the card was seen against a grey square surrounded by other greys. The card now appeared bright *pink* though the spectral lights on it had remained untouched. This was to be expected, for initially the card matched the square, so when the red component was removed from the square it should be seen in excess on the card. This expectation proves exact, for if the RGB energies on the card are measured when matched with the square illuminated by: (*a*) the white projection only; (*b*) the red only; (*c*) both together, it is found for each of R, G and B that the energies in (*c*) are the sum of those in (*a*) and (*b*).

It is a well-known feature of the Land picture that its general appearance is little affected by the relative intensity of the two projection lights. Though the 'warmth' of the picture is altered, the red square and the green square are still so named after the ratio of the projection lights has been greatly changed. What happens in these circumstances to the *RGB* proportions on the matched comparison card ?

Experiment 2. The card was placed in front of a green square and matched, the red and the white energies falling on the *square* being measured by interposition of a photomultiplier tube. Now the projection image was shifted across the screen so that, for example, a red square lay behind the card. The two projection intensities were altered so that the energies received on this (still red) square were the same as they had been on the former green one. The unchanged light falling on the interposed card matched perfectly this new square, but now it looked red, not green. Every square with red-white energies adjusted to these fixed values was matched by the fixed card which assumed the colour of each square in turn when viewed in that particular location in the picture.

Conclusion from Monocular Matches

The results are simple and 'classical', being in accord with the law which Grassmann[5] enunciated more than a century ago, namely, that two lights which appear identical in one situation appear so in all. The *RGB* mixture required to match any square is simply the sum of the energies required to match the white component and the red component falling separately on that square. The match is therefore dichromatic, and the projection of two lights, so far from exhibiting 'every colour', leaves enormously more colours unrepresented than those capable of being displayed. This may readily be seen by making a perfect *RGB* match for any Land projection and then altering any one of the *RGB* components to any extent. Every one of the colours so formed is neither produced nor producible by the Land projections employed whatever their ratio or intensity. If we believe that there are three cone pigments in the human eye, all the foregoing matching results may be shown to follow as an inescapable logical consequence.

A fine painting may evoke so realistic an appearance of depth that it is hard to believe that all we see is a flat sheet. There is a sense, then, in which we may say that three dimensions have been represented by only two, but the sense is artistic rather than scientific. It is in a similar sense that we may say that Land's art has evoked the three dimensions of colour.

Complexities in Binocular Matches

We have seen with monocular matches that when considerations are restricted to judgments of identity or non-identity, the relations are precisely of the kind that would be found if the eye were replaced by three photocells each with different spectral sensitivity. So much is entirely expected ; what is surprising is our sensation, for example, that a fixed light looks now red, now green. What is bewitched by Land's magic lantern ? Is it the eye—or is it the brain ? Perhaps when a red light appears green the eye is still sending the normal 'red signal' up the optic nerve, but the brain, confused by messages from the retinal surround, decodes the signal incorrectly. This suggestion can be shown to be wrong. For if it were right a similar normal 'red signal' coming to the brain from the other eye would also be decoded incorrectly in precisely the same way, and hence look the same. (For simplicity of treatment the 'brain' is here considered as a single decoding device operating equally on the messages from each optic nerve and delivering a decoded output which stands in one-to-one relation with visual sensation.)

Experiment 3. One may look with the left eye at the green square in the Land projection, and with the right eye down a tube observing nothing but a card illuminated by the *RGB* mixture from the monochromator. If the mixture on the card matches the green square by monocular superposition it is generally found not to match at all viewed binocularly : the square looks green, the card looks pink, so the left eye cannot be transmitting a normal red signal.

The signal which is being transmitted will be the same as that in the right eye when a binocular match has been established. But Land's colours in general cannot be matched by any spectral mixture seen in the other eye. The browns, olives and greys have too much black in them. This difficulty was overcome by surrounding the card presented to the right eye by a white background of about the colour and intensity of the white Land projection alone.

Experiment 4. These matches were not very easy to make but were reasonably repeatable, and the *RGB* mixture when later observed alone corresponded roughly with the remembered appearance of the particular square matched, so the green-appearing square was matched by a green part of the spectrum (blackened by contrast to the white surround). The particular spectral wave-length corresponding to the *RGB* mixture could be found from the colour triangle of Wright[6] whose primary wave-lengths we have adopted.

Experiment 2 was repeated but with binocular matches made against various squares, each adjusted to receive a fixed energy from the white and from the red projector. The results were quite different from those of Experiment 2, where the match was monocular. Each square now needed a different *RGB* mixture and the (darkened) spectral colour resulting corresponded fairly well to the coloured appearance of each square as seen in normal viewing.

When a binocular match has been established it is plausible (though not certain) that the signals in the two optic nerves are the same. Therefore the binocular match will measure that part of the 'Land effect' which is due to unusual encoding by the eye. It cannot give information about that part which is due to unusual decoding by the brain, since this will be the same for both nerves. That abnormal decoding may be involved, however, is seen from the following experiment.

Experiment 5. A narrow tube is pointed at the Land figure so that on looking through the tube all that is seen is part of one square. First the whole picture is viewed and that particular square noted ; then on looking through the tube its colour is seen unaltered, nor is any change seen on continued observation.

Here is a paradox, for when this experiment is repeated with various squares, each adjusted to receive the same red and white projection energies as in Experiment 2, we have the extraordinary situation in which the eye looks through the tube at an isolated and identical red-white mixture and sees this as a colour which persists (though with dwindling conviction) with a hue which may vary greatly depending upon the past history. It will in fact be the colour of the square as seen previously in the full picture. Though this colour persists—or rather is not seen to change so long as one looks down the tube at the unchanging square, it alters instantly on glancing at a new surround and back down the tube. This persistent memory and sudden change of judgment suggest activity of brain, not eye, and binocular comparison confirms it.

Experiment 6. A binocular match was made as in Experiment 4, and suddenly the surround to the small square was removed by interposition of the tube at the left eye. The binocular match was immediately upset and for restoration needed an *RGB* mixture close to the proportions for the monocular match, though the white surround to the comparison field made a perfect match unobtainable.

This experiment shows that when the surround field is abolished in the left eye the encoded message (as judged by the colour match in the right eye) changes at once to the normal 'pink message'. But it is not seen to change if viewed without binocular comparison. The decoding mechanism in the brain, therefore, either is insensitive to this change of message or it deliberately compensates for it. The first possibility is excluded by the fact that a much smaller alteration in nerve message produced by slightly changing the red/white ratio of the projection lights is easily detected.

We thus arrive at the surprising conclusion (though familiar to psychologists as an example of 'colour constancy') that when the same square is observed through the tube and in the open, the brain in some way recognizes the identity and in its decoding exactly compensates for the change in the message which it receives : and, indeed, when a green square is matched binocularly and then has its surround abolished, the sensation experienced is not that the square goes pink, but that the comparison card suddenly gets more green—at least if one's attention is fixed primarily upon the square. But I am trying to avoid speaking of my sensations !

Conclusions

This article reaches some over-simplified and rather obvious conclusions about the difficult subject of colour appearance.

(*a*) It is easier to analyse by colour matching than by colour naming.

(*b*) Monocular matches are simply the sum of the matches made with each component of projection alone. They follow Grassmann's law and the logic of three cone pigments.

(*c*) Colour appearance may depend on the surrounding field and its past history. These alter the encoding of nerve messages by the eye, and decoding by the brain.

(*d*) The extent of the former may be measured by binocular matches, the extent of the latter by memory matches.

(*e*) The eye–brain model postulated is crude, but it reveals some of the complexity which underlies the sensation of colour.

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OBITUARIES

Prof. P. G. H. Boswell, O.B.E., F.R.S.

FEW geologists have accomplished more in the face of chronic ill-health than P. G. H. Boswell, emeritus professor of geology of the Imperial College of Science and Technology, London, who died on December 22, 1960, at Ruthin, North Wales, at the age of seventy-four. Not only did he contribute close on 150 publications, including several memoirs and books, but also he was for long vigorously engaged in teaching and administration at the Universities of Liverpool and London, in the conduct of learned societies, and in proffering geological advice to various industrial and public undertakings.

Born at Woodbridge, Suffolk, in 1886, he developed during adolescence such an absorbing interest in the geology of his native county that in 1912 he abandoned teaching in an elementary school so as to concentrate on geology by studying under W. W. Watts at the Imperial College. While there he continued his researches on the Pliocene–Pleistocene succession in East Anglia and helped to correlate Early human phases with those of the Glacial Period, besides devoting much attention to the Chalk and to an assessment of the magnitude of the Eocene unconformity in the London Basin.

It was while investigating the Tertiary rocks that his thoughts turned to the possibility of using their detrital mineral assemblages for correlating particular horizons. This led him to specialize in the quantitative and qualitative aspects of sedimentary petrology which were to prove so helpful in deciphering the palæogeography, tectonics and climatic conditions of past epochs, and so valuable in connexion with the search for glass-making, moulding and refractory sands during and after the First World War. Shortly after his appointment to the chair of geology at Liverpool in 1917, Boswell started his protracted researches, lasting more than twenty-five years, into the stratigraphy and tectonics of the Silurian rocks of the Denbighshire Moors. Even though some of his structural interpretations have been assailed, there is no doubt that most of his observations, gathered together in a volume on the *Middle Silurian Rocks of North Wales* (1949), will stand the test of time. Always actively interested in the applications of geology to civil engineering, his advice during the planning and construction of the Mersey road tunnel contributed in no small measure to the success of that major project.

In 1930 he was invited to succeed Watts as professor of geology at the Imperial College, but his tenure of the chair was comparatively brief, for he was compelled to resign eight years later because of ill-health. Nevertheless, he continued his work in North Wales and his consulting practice until 1953 ; thereafter, though gravely incapacitated, he still wrote a number of papers on thixotropy, greywackes and allied subjects, ending with a treatise on muddy sediments, to be published posthumously.

Boswell had been president of the Geological Societies of Liverpool (1921–23) and of London

(1940–41), of the Prehistoric Society (1936), and was successively general secretary (1931–35) and general treasurer (1935–43) of the British Association, besides being president of Section C (Geology) in 1932. Other honours included the Bigsby Medal of the Geological Society of London (1928), election to the Royal Society (1931), and honorary membership of many scientific societies at home and abroad; he was made O.B.E. in 1918. He will be remembered with affection and esteem by all his former colleagues and by generations of students, not least by those he inspired at Liverpool during the hey-day of his career.

DAVID WILLIAMS

Prof. E. T. Bell

E. T. BELL, who died in 1960, was born at Aberdeen in 1883. His schooldays were spent in Great Britain, but he attended university in the United States, where he spent the whole of his adult life. He occupied a number of university posts, culminating in his tenure of the chair of mathematics at the California Institute of Technology, Pasadena, 1927–53.

As a schoolboy, Bell came under a mathematical teacher of rare distinction, E. M. Langley, of Bedford, and on Langley's death wrote with warm appreciation of being shown something of the mode and content of modern mathematics by an inspiring teacher, and in particular of being introduced at school to the theory of numbers and to elliptic functions. His research work was mainly in these fields, and he made significant contributions to the classical theory of numbers, the field of rational arithmetic and of Diophantine analysis.

Bell's name, however, was well known outside the restricted circle of professional mathematicians, by the series of books, from about 1930 onwards, in which he endeavoured, with substantial success, to explain modern mathematics to the intelligent layman. Of these books, three deserve special mention. In *Mathematics, Queen and Servant of Science* (1952), a conflation of two earlier volumes, he described the dual nature of mathematics, its remoteness from prevalent crude materialism and its extraordinary knack of becoming vitally relevant to material concerns. If there are two cultures, mathematics, belonging to both, is the essential bridge between them. *Men of Mathematics* (1937) relates the outstanding mathematical discoveries of the ages to the men who made them; sufficient personal detail is given to allow the reader to see mathematicians as human beings, but emphasis throughout is on their contributions to mathematics. Something of the same theme runs through *The Development of Mathematics* (1940), which is not an encyclopædic history of the subject, but a narrative of carefully chosen main trends, themselves presented through typical major episodes; it is a graphic map of the main roads, and omission of byways and dead ends is deliberate and justifiable. Even so, the amount of information and opinion packed into 550 pages is extraordinary.

Bell's facts are almost always sound, his style is clear and exuberant, his opinions, whether we agree with them or not, are expressed forcefully, often with humour and a little gentle malice. He was no uncritical hero-worshipper, being as quick to mark the opportunity lost as the ground gained, so that from his books we get a vision of mathematics as a high activity of the questing human mind, often fallible, but always pressing on in the never-ending search for mathematical truth.

T. A. A. BROADBENT

NEWS and VIEWS

Royal Geographical Society: Medals and Awards

H.M. THE QUEEN has approved the award of the Royal Medals for 1961 of the Royal Geographical Society as follows. *Patron's Medal:* Mikhail M. Somov, deputy director, Arctic and Antarctic Research Institute, Leningrad, for polar exploration and research; *Founder's Medal:* John Bartholomew, editor, *The Times Atlas of the World,* for contributions to cartography.

The Council of the Royal Geographical Society has made the following awards. *Victoria Medal:* Prof. W. William-Olsson, Stockholm School of Economics, for contributions to economic geography; *Murchison Grant:* Prof. K. M. Buchanan, University College, Wellington, New Zealand, for contributions to economic geography; *Back Grant:* Eric H. Brown, University College, London, for geomorphological research; *Cuthbert Peek Grant:* Martin W. Holdgate, Scott Polar Research Institute, Cambridge, and leader of the Royal Society's Expedition to southern Chile; *Gill Memorial:* Cuchlaine A. M. King, University of Nottingham, for glaciological and coastal research; *Mrs. Patrick Ness Award:* R. M. L. Mason and A. R. Hanbury-Tenison, for their trans-continental exploratory journey in South America.

Statistics at Birkbeck College, London: Prof. D. R. Cox

THE establishment of a chair in statistics at Birkbeck College, University of London, marks the fulfilment of a scheme the authorities have had in mind for some few years of establishing statistics at the College. Birkbeck College is a college of the University of London reserved for students who are in full-time occupation, so that the majority of the students attend only in the evening. In the past, the object of the College was to provide a university education for students who, for financial or other reasons, were unable to secure this in the normal way; but the development of university education in Britain has introduced many new elements. It is now much easier for a student to secure full-time education; on the other hand, there is now a need for many more men with qualifications in two allied subjects, and the College is finding that more and more of its students are fitting into this class. In particular, there are many who have taken degrees in mathematics who wish to obtain a qualification in statistics, and in developing this new department, the College has the needs of these students particularly before it. The department will provide courses for postgraduate and undergraduate students.

Dr. D. R. Cox, reader in statistics in the College, has been appointed to the new chair. His interests are the scientific and technological applications of statistics and probability, and the theory of statistics. His published work includes a book on the *Planning of Experiments* and he is joint author of a book on the theory of queues to be published shortly. His present research interests include prediction theory, various applications of the theory of probability in operational research, and the design and statistical analysis of experiments.

Division of Pure Physics, National Research Council of Canada: Dr. D. C. Rose, O.B.E.

DR. D. C. ROSE has been appointed director of the Division of Pure Physics, National Research Council of Canada. Dr. Rose was born in Prescott, Ontario, in 1901, and gained his B.Sc. degree in 1923 and his M.Sc. degree in 1924 from Queen's University. He was awarded an 1851 Scholarship for study, under Rutherford, at Cambridge, where he obtained his Ph.D. degree in 1927. After continuing his work at the Cavendish Laboratory until 1928, he spent 1928–29 at the H. H. Wills Physical Laboratory, Bristol, and 1929–30 as a lecturer at Queen's University. In 1930 he joined the Division of Physics of the National Research Council of Canada. During the Second World War, Dr. Rose was seconded to the Department of National Defence, first as scientific adviser to the Chief of the General Staff, during 1943–45, and later, during 1945–47, as chief superintendent of the Canadian Armament Research and Development Establishment at Valcartier. Returning from war-time duties to the National Research Council, he established a laboratory in cosmic-ray physics, which he still heads. For his contributions to the war effort, Dr. Rose was made O.B.E. Dr. Rose was elected a Fellow of the Royal Society of Canada in 1936; he has served the Society for several years as secretary of Section III. He is a past president of the Canadian Association of Physicists, a member of the American Physical Society and a member of the Engineering Institute of Canada.

Building Contracts for Universities

IN his report to the University Grants Committee reviewing the methods used by universities of contracting and of recording and controlling expenditure, Sir Arthur Rucker has some fourteen conclusions relating to the recommendations of the Gater Committee. The appointment of a small project sub-committee for each major building project is generally desirable and now usual, and this sub-committee should have in mind both the user's need and the needs of economy. There is occasionally difficulty in finding suitable persons with business and financial experience from outside a university to serve on the council and committees of the university, but every effort should be made to do so. Universities should keep their needs for staff under review to ensure that they and their committees have the advice and assistance they require to maintain proper control in their expanded building programme. No university should include in the list of firms invited to tender for a building contract any firm to which, if it were the successful competitor, the university would not desire to award the contract. Undue economy in capital costs at the expense of maintenance costs would be regrettable, but in a well-designed building it should be possible, within a reasonable over-all cost, to use materials and methods that will avoid excessive future maintenance costs.

It is important that all possible means should be sought both to make the maximum use of costly university buildings and to avoid planning new buildings on too low a standard of student use. Economy will always best be served by avoiding a break in the continuity of construction, and this should normally be possible in the present enlarged building programmes. The control exercised by universities and their committees and by the University Grants Committee over variations in plan after tenders have been invited is firm, and it is essential it should remain so. The normal practice of universities is, and should continue to be, to invite competitive tenders on a fixed price basis; in suitable cases negotiated contracts may be advantageous. Methods of letting sub-contracts have varied, but none of the methods used appears to have caused difficulty. The selection of sub-contractors before the letting of the main contract is, however, desirable. It is usually necessary to specify the date by which a contract is to be completed in the tender form, but the date should not be a matter for competition. The present practice of providing a sum for contingencies not exceeding 3 per cent appears to be generally satisfactory to universities, and is satisfactory so long as it is used for true contingencies and not for changes in plan. Architects should be required by all universities to make regular written reports to the responsible committees at least once a month, and in all universities the responsible committee should be provided at regular intervals with figures showing the estimated final commitment on each major contract. The general features of the Rucker Report are discussed elsewhere in this issue of *Nature* (p. 427).

Student Wastage in Australia

ACCORDING to a *Times* correspondent, Prof. R. Le Fèvre, professor and head of the School of Chemistry in the University of Sydney, has stated that that University failed 1,000 first-year students who sat for chemistry examinations in 1960. Prof. Le Fèvre said that if failures in other science subjects were included, the failure-rate would be about 50 per cent. There were too few lecturers and too many students. "We have a British university system imposed on an American schooling system. What we need is something like the American junior college system or the British sixth form which provides a suitable bridge between high school and university." Prof. Le Fèvre said that some students did not know how to work and some just wanted to have a good time. The only solution appeared to be the introduction of some kind of pre-selection system.

There seems to be little doubt that, so far as science, in any event, is concerned, the Australian system needs serious overhauling. Far too much time, money and space are wasted in the training of first-year students. This is, of course, to the detriment of standards within the university itself; but even worse is the fact that such student failures have apparently wasted one year of the most formative phase in their lives.

International Laws on Nuclear Incidents

THE Convention on Third Party Liability in the Field of Nuclear Energy, of July 29, 1960, was signed at Paris by the Governments of the West German Federal Republic, Austria, Belgium, Denmark, Spain, France, Greece, Italy, Luxembourg, Norway, the Netherlands, Portugal, the United Kingdom, Sweden, Switzerland and Turkey. The text of the Convention, which has now been published by the Organization for European Economic Co-operation, contains 24 articles (Pp. 67. Paris: O.E.E.C., European Nuclear Energy Agency, 1960). Its scope is limited to risks for which common law rules and practice are unsuitable, and it does not apply to nuclear incidents occurring in the territory of non-contracting States or to damage suffered in such territory. Its special regime applies only to nuclear incidents occurring at or in connexion with certain nuclear installations or in the transport of nuclear substances as defined. Risks which arise in respect of radioisotopes used for any industrial, commercial, agricultural, medical or scientific purpose are excluded, once the radioisotopes are applied for those purposes, as such risks are not exceptional: nuclear propulsion and nuclear fusion are also excluded, for different reasons. All liability for installations is channelled on to the operator of the nuclear installation where the incident occurs, while liability for incidents in transport falls either on the carrier or on the operator of the nuclear installation concerned. There are detailed provisions determining the kind of damage which will be compensated, and maximum liability in respect of any single incident has been fixed at 15 million European Monetary Agreement units of account, and rights of compensation are extinguished ten years from the date of an incident if no action has been brought before a competent court.

Detection and Identification of Underground Explosions

In connexion with the work of the conference on nuclear test control at Geneva, the Government recently announced its intention to carry out a programme of seismic research bearing on the detection and identification of underground nuclear explosions. This programme is now being planned in detail by the U.K. Atomic Energy Authority and suitable areas for the siting of seismic measuring instruments are being investigated. The research programme will involve the firing of conventional explosive charges, but no nuclear explosives will be used. Some of the firings will probably take place in disused mines and quarries: others may take the form of charges dropped at selected points in the Channel from naval vessels. An operational centre for seismic work is being established at a converted country house a few miles from Aldermaston.

The Field Studies Council

A wide range of courses dealing with many topics has been arranged by the Field Studies Council for 1961. At certain periods, normally March–Whitsun, there is great pressure on accommodation at the Centres and priority is given to members.

The Easter courses for schools are now designed for second- and third-year sixth-form pupils only. For pupils in secondary modern schools, a number of countryside courses have been arranged. The programmes again include a choice of courses specially designed for teachers in primary, secondary modern and grammar schools. At all times, even when courses have been arranged, places are kept for artists, amateur naturalists and other individuals or groups who wish to organize their own programme or to pursue studies independently, or in a less formal way than is suggested by the word 'course'. The Council has at its disposal a small bursary fund which is available to reduce the fee charged in individual cases of merit where grant-aid is needed but is not obtainable. Among the more unusual courses is that at Dale Fort for teachers dealing with marine biology and oceanography as well as "Man in Pembrokeshire", a course in historical geography. At Flatford Mill a course is to be run for amateur entomologists introducing them to field techniques in insect ecology. Juniper Hall has a course on slugs and snails, while Malham Tarn has a special course on karstic landforms as well as one on spider ecology. Students of geomorphology will find a special course at Preston Montford, while Slapton Ley provides an introduction to field archæology. Details of these, as well as the more routine, courses may be obtained from the Publicity Secretary, Field Studies Council, Ravensmead, Keston, Kent.

Biology, Psychology and Belief

The fourteenth lecture in memory of Sir Arthur Eddington was delivered in Cambridge on November 3 by Dr. W. H. Thorpe. Dealing with the relationships between the biological sciences and religious belief, Thorpe's lecture takes a rightful place among the more outstanding of its predecessors. To this lecture Thorpe has brought an honesty of purpose which should be of considerable help to biologists confronted with difficulties in reaching some general system of faith. There is also a clarity of thinking which is concerned never to confuse but to paint a world of the spirit as revealed to Thorpe. That man is a biological, as well as a spiritual, organism is well brought out, as is the conviction that no system of faith or belief has any ultimate prospect of securing the loyal and lasting adherence of educated men in the twentieth and later centuries unless it is firmly rooted in biological fact. The lecture has now been published as a booklet and should appeal to readers of all disciplines concerned with the nature of spiritual belief (Pp. 60. Cambridge: At the University Press, 1960. 4*s.* 6*d.*).

Rock Shelters of the Lower Murray River

A recent issue of the *Proceedings of the Royal Society of Victoria* contains an interesting account of an excavation undertaken at Fromm's Landing on the Lower Murray River under the leadership of D. J. Mulvaney, of the University of Melbourne (New Series, **72**, Pt. 2. Victoria: The Royal Society, 1960). A rock-shelter, facing east and well suited for habitation, was explored and no less than ten different levels determined. Not enough attention has been paid by prehistorians to recent work in Australia. Maybe the finds in various regions of Africa have too exclusively engrossed their attention. Australian Stone-Age sites have been dismissed as early native. The carbon-14 method of dating has many pitfalls and cannot always be entirely relied on unless many samples from a culture-level (and not all from the same site) have been examined. But the chances of reasonable accuracy are increasing as workers in the field are learning what precautions must be taken in procuring samples, and in the laboratory new techniques are being developed. The carbon-14 results for the lower levels at Fromm's Landing suggest a date some 4,000 years ago and one which therefore falls well within most prehistorians' periods.

Fromm's Landing is not the only site on the Lower Murray which has been explored: there is the Devon Downs shelter, with which Fromm's Landing can be compared, and there are others. As is to be expected, there are differences in the finds, both implements and fauna, as one proceeds from the top layer to layer 10. But these differences do not appear to be startling. Actually the site is not prolific in artefacts, but adzes, 'pirri' points (small, somewhat thick-based arrow-head-like affairs), microliths of various kinds and bone objects have been collected, as well as the bones of various mammals, reptiles, birds and fish. Naturally, too, shells, both univalves and bivalves, occur. On the walls of the shelter there are some rough markings, but these are scarcely worthy of the designation 'art'. The article is illustrated and the numbers under each illustration of a tool refer to accounts of them given in the text. There are, however, several such accounts referring to numbers which do not appear among the illustrations. Has a page of illustrations by accident been left out? The author is to be congratulated on an excellent piece of work. Should some of these habitation sites in Australia really date back to several thousands of years ago, it is high time geologists there took the matter up and determined a sequence of climate changes for the various areas in the continent. It is scarcely conceivable that no perceptible changes have taken place over so long a period. The correlation of such a climate sequence with the study of the stone industries from the various levels would mean an important addition to knowledge.

Birds of the Philippine Islands

It is known that the size of an island may be an important factor in determining certain characteristics of its avifauna. Recently, A. L. Rand and D. S. Rabor have directed attention to "a surprising but apparently very real phenomenon in the Philippines", namely, that species commonly restricted to higher altitudes may live at lower ones on small islands (*Fieldiana*: Zoology, **35**, No. 7, 221. Chicago: Chicago Natural History Museum, 1960. 4 dollars). Thus the fruit pigeon *Ptilinopus merrilli* is a mountain bird on Luzon, but lives at sea-level on the adjacent small Polillo Island; and there are similar cases. The brush cuckoo *Cacomantis variolosus* is of special interest because it lives on the mountains of Zamboanga, a large island of which the lower levels are inhabited by the related *C. merulinus*; on Siquijor (90 square miles) only *C. variolosus* is found, living far below what is regarded as its normal range. This observation may be considered with the known fact that there are species of wide distribution in south-east Asia which live only on small islands, the nutmeg pigeon *Ducula bicolor* being an example of this in the Philippine archipelago. The speculation is made whether a restricted montane habitat surrounded by lowlands may not in some way be equated to a small insular habitat surrounded by sea.

New Species of *Chaetacis* from South America

While working on collections of spiders in the British Museum (Natural History), Arthur M. Chickering of Albion College, Albion, Michigan, found numerous examples of the genus *Chaetacis* from South America. Chickering separated out specimens which appear to represent five new species, thus bringing the total number of known species to twelve. Two of the new species, which he describes in the August issue of *Annals and Magazine of Natural History* (2, No. 20; 1959), are represented only by males which have not previously been described, although apparently Eugene Simon saw one when he established the genus in 1895. The types are to be deposited in the Department of Zoology, British Museum (Natural History).

Measurement of Molecular Weights

An easy-to-operate device for the accurate determination of molecular weights of unknown materials in solution has been developed by R. A. Pasternak and Leonard A. Cavanagh, and is described in the November–December issue of *Research for Industry* (12, No. 6; 1960). This efficient analytical tool is also valuable for research on interactions of compounds in solution. The key to the instrument is a thermostatically controlled chamber, the atmosphere of which is saturated with the vapour of a solvent. A probe composed of two bead thermistors is enclosed. Drops of the solvent and of the sample solution are applied to the beads through one of the six syringe assemblies of the unit. At any given temperature the vapour pressure of a solution is lower than that of the solvent. Therefore, the solution drop warms up (by condensation of minute amounts of the solvent) until pressure balance between the two drops is attained. The temperature rise, detected by the thermistor probe, is about proportional to the 'osmotic concentration' of the sample solution. When the instrument is calibrated with solutions of known concentration, it is then possible to calculate the molecular weight of the unknown solution. The new instrument, which was developed for Mechrolab, Mountain View, California, has great advantages over present molecular weight devices. Because of the syringe assembly, it does not have to be opened after each sample. Indeed, duplicate readings can be taken on fresh samples within 1–5 min. An accuracy of 1–2 per cent is claimed. Moreover, only very small amounts of the sample are required for testing.

Fiftieth Anniversary Meeting of the Biochemical Society

The fiftieth anniversary meeting of the Biochemical Society is to be held during March 27–29, and will be centred on University College, London. The meeting will include a symposium and a memorial lecture. The symposium is to be on "Structure and Synthesis of Macromolecules" (March 27–28), and will be opened by Prof. F. G. Young (Cambridge). Among the other speakers are: Prof. P. Doty (Harvard); Prof. J. N. Davidson (Glasgow); Prof. E. L. Hirst (Edinburgh); Prof. W. Z. Hassid (Berkeley); Dr. M. F. Perutz (Cambridge); Prof. E. L. Smith (Salt Lake City); Prof. J. Monod (Paris). The third Hopkins Memorial Lecture will be given by Sir Hans Krebs, and is entitled "The Physiological Role of Ketane Bodies" (March 28). The annual general meeting and anniversary dinner will take place on March 29. Further information can be obtained from the honorary secretary, Dr. W. J. Whelan, Lister Institute of Preventive Medicine, Chelsea Bridge Road, London, S.W.1.

City of Liverpool College of Technology

It is announced that the following are to become honorary associates of the College: Mr. F. W. Adams (secretary and registrar of the Pharmaceutical Society of Great Britain); Sir Edward Bullard (Department of Geodesy and Geophysics, University of Cambridge); Mr. E. Le Q. Herbert (president of the Royal Institute of Chemistry); Sir Alan Tod (president of the Liverpool and District Scientific, Industrial and Research Library Advisory Council).

University News: McGill University

THE research groups and weather observatory which have been responsible for the meteorology carried out at McGill University have now been supplemented by a Department of Meteorology, consisting of Profs. J. S. Marshall and F. K. Hare, Associate and Assistant Profs. R. H. Douglas, W. Hitschfeld, S. Orvig and B. W. Boville. In the undergraduate curriculum, four continuation courses in meteorology are available to the general programme toward the B.Sc. degree. For the postgraduate programme leading to the M.Sc., the normal admission requirement is a bachelor's degree with honours in physics, and there is no prerequisite meteorology. Candidates proceeding from master's degree to Ph.D. will continue as in the past to be primarily the concern of the research groups, with the possibility of some helpful backing from the Department. Prof. Marshall retains his title and duties as Macdonald professor of physics, and Prof. Hare continues as professor of geography and chairman of that Department. Profs. Boville and Orvig are known for their work in the Arctic Meteorology Research Group, and Profs. Douglas and Hitschfeld in connexion with the Stormy Weather Group. Until the present session, Dr. Douglas and Mr. Boville have been at McGill as members of the Canadian Meteorological Service, to which they have made important contributions. By the end of the present session their formal connexion to the Service will have been severed, but there will be close and continuing co-operation between this first Canadian University Department of Meteorology and the Canadian Meteorological Service.

Sheffield

Two new lectureships have been announced: Mr. A. Usher is to be lecturer in forensic medicine; and Mr. H. Merskey, to be lecturer in psychiatry.

Swansea

DR. J. D. WESTON, senior lecturer in mathematics, King's College, Newcastle upon Tyne, has been appointed professor of pure mathematics, to succeed Prof. R. Wilson, who retires at the end of the present session.

A one-year course, leading to a College diploma in chemical engineering, will be instituted in the session 1961–62. It will be open to students holding an honours degree, or equivalent qualification, in a branch of physical science or engineering, and the content of the course will be adjusted according to the background of the individual student. It will consist of lectures, tutorials, laboratory and design work, extending over a full academic session. Applications should reach the Registrar, University College, Swansea, by June 30.

Announcements

SIR JOHN COCKCROFT, master-designate of Churchill College, Cambridge, and part-time member of the U.K. Atomic Energy Authority, has been awarded the 1961 Atoms for Peace Award, for his contribution towards the application of atomic energy to the production of electricity in the United Kingdom. The Award, established as a memorial to Henry Ford and his son Edsel, consists of a gold medal and 75,000 dollars.

PROF. EPHRAIM KATCHALSKI, head of the Biophysics Department, Weizmann Institute of Science, has been awarded the Rothschild Prize for Research for 1960, in recognition of his work on the structure of poly-amino-acids.

MR. S. N. MITRA, of the Research Department, All India Radio, New Delhi, has been awarded the 1960 prize of the Council of the Institution of Telecommunication Engineers of India for his paper, "Magneto–Ionic Triple Splitting over Delhi".

PROF. W. T. ASTBURY, professor of biomolecular structure in the University of Leeds, has been elected the fourth recipient of the T. Duckett Jones Memorial Award. This is a medical award of 6,500 dollars administered by the Helen Hay Whitney Foundation, and it will be presented to Prof. Astbury at Princeton, New Jersey, on October 7, 1961.

MR. E. LL. HARRY, at present regional controller of the East Midland Region of the Ministry of Agriculture, Fisheries and Food, is to succeed Mr. C. D. Ross as regional director of the National Agricultural Advisory Service, South Western Region, when the latter retires on March 9.

A SYMPOSIUM and instrument exhibition on "Automatic and Semi-Automatic Methods in Analysis and Control" will be held at the Medway College of Technology on March 16. Further information can be obtained from the course organizer, Dr. D. T. Burns, Department of Science, Medway College of Technology, Maidstone Road, Chatham, Kent.

THE Faraday Society is organizing a general discussion on "Radiation Effects in Inorganic Solids" by invitation at the Centre d'Études Nucléaires de Saclay, Gif-sur-Yvette (S.-et-O.), during April 11–12. Further information can be obtained from the Assistant Secretary, Faraday Society, 6 Gray's Inn Square, London, W.C.1.

AN intensive short course in elementary plasticity is being organized by the Imperial College of Science and Technology, in the Department of Mechanical Engineering, during April 10–14. Further information can be obtained from Dr. J. M. Alexander, Department of Mechanical Engineering, Imperial College of Science and Technology, Exhibition Road, London, S.W.7.

THE Faraday Society is holding an informal discussion on the "Mechanisms of the Gas-Phase Reactions of the Halogens and their Compounds" in the Chemistry Department, University College, Cardiff, on March 28. The programme will be divided into two sections: (*a*) oxidation processes; (*b*) reactions of elementary halogens. Further information can be obtained from Dr. J. H. Thomas, Chemistry Department, University College, Cathays Park, Cardiff.

ERRATUM. In *Nature* of January 28, p. 275, concerning recent changes in administration and organization of the Akers Research Laboratory, Imperial Chemical Industries, Ltd., it was erroneously stated that Dr. Chatt will continue in charge of the Organic Chemistry Department; Dr. Chatt is in charge of the Inorganic Chemistry Department.

NEW LABORATORY OF LONDON TRANSPORT

By A. T. WILFORD

Director of Research

A NEW laboratory situated on London Transport's Chiswick Works Estate was officially opened by Mr. A. B. B. Valentine, chairman of the London Transport Executive, on December 5. It will continue the function of providing a scientific service available to all departments of London Transport and will permit an expansion of these facilities, with particular reference to engineering problems. At the same time, the small laboratories concerned with essential day-to-day chemical control at the three generating stations are being retained.

The history of the Chiswick Laboratory goes back to more than forty years ago, when a small laboratory was established by the former London General Omnibus Co. at Farm Lane, Fulham, primarily for testing petrol and lubricating oils. It was transferred to Chiswick Works, then newly opened, in 1,921. The scope of its activities, mainly in the chemical field, gradually extended, and improved accommodation was provided in 1931. By the time that the London Passenger Transport Board was formed in 1933 the laboratory was beginning to provide a service also for departments of the undertaking other than those concerned with road vehicles, and in 1949 it became officially recognized as London Transport's Central Laboratory. Immediately prior to its transfer to the new premises, the Laboratory was housed in portions of four scattered buildings and three temporary huts on the Chiswick Works Estate.

The new Laboratory premises (Fig. 1) comprise a steel-framed two-storey building with an adjacent single-story annex, the total floor area being 27,000 sq. ft. Metallurgical, physics and engineering activities are housed on the ground floor, the chemical sections and general administration being located on the first floor. The single-storey annex contains a shop for manufacturing special chemical mixtures, also a pilot plant shop. Its roof is linked by a bridge to the first floor of the main building and serves as a site for weather exposure testing of paints. (A second exposure testing station, where conditions are somewhat more adverse, is maintained on the roof of the Executive's Upton Park Garage.)

Except in the engineering laboratories and workshops, the services, which include gas, electricity,

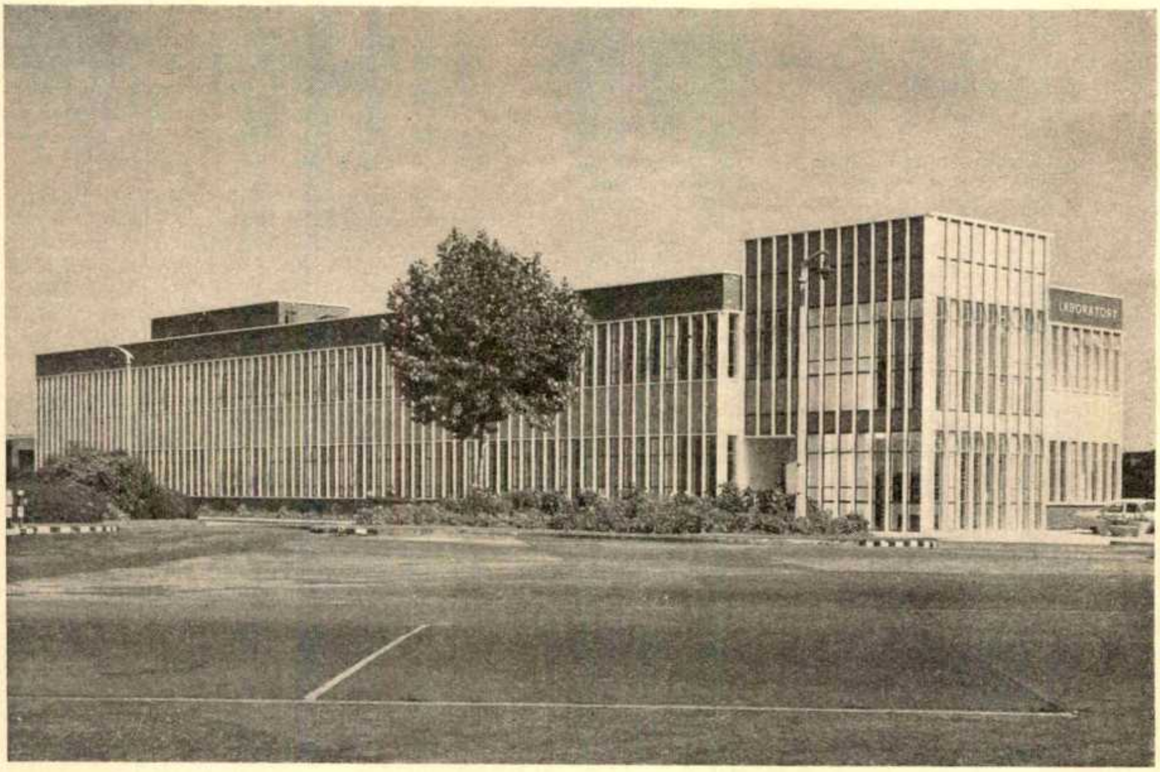

Fig. 1. New Laboratory of London Transport at Chiswick

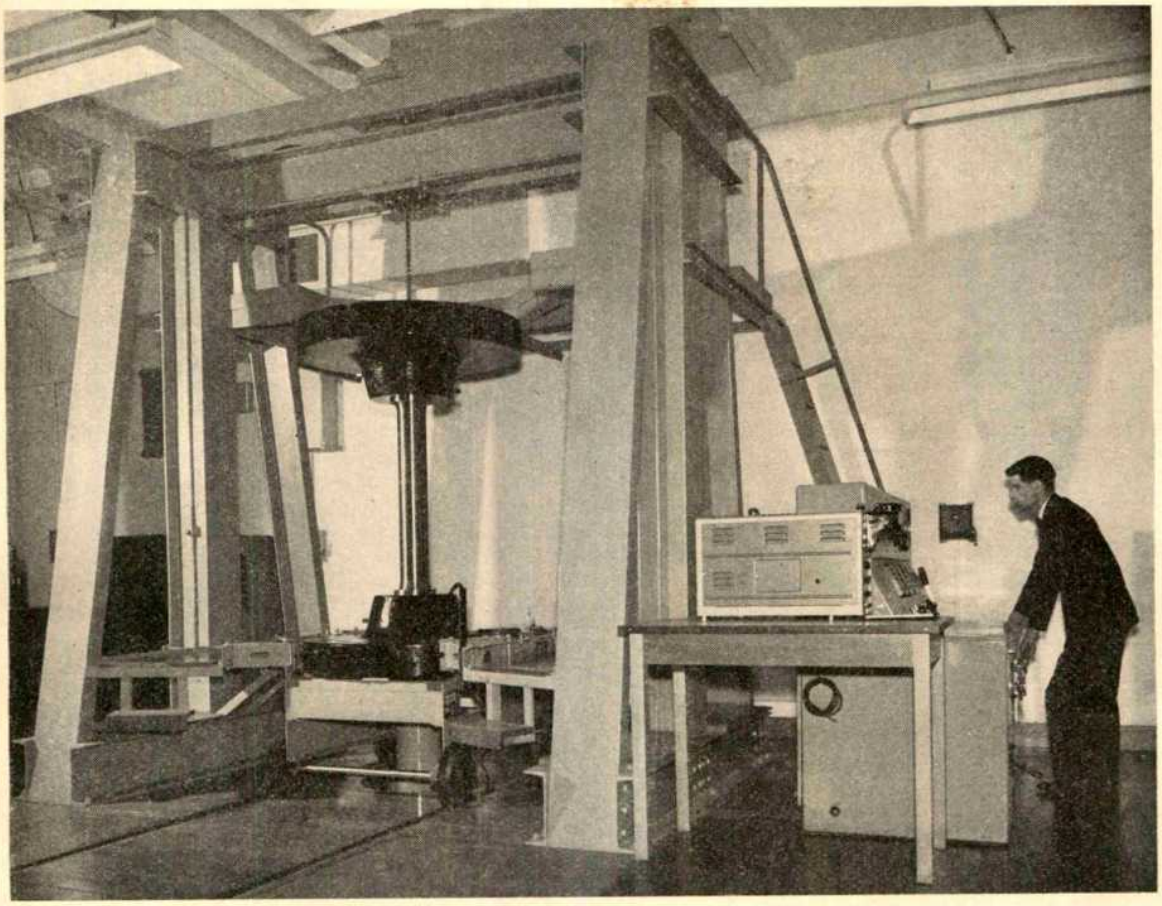

Fig. 2. Fatigue testing machine developed in the University of Nottingham

steam, hot water and pressurized cold water, are carried above removable ceiling panels, and the use of ceiling heating permits the most efficient use of laboratory floor space. Air extracted through fume cupboards, etc., is replaced by means of a plenum system which introduces air at a pre-adjusted temperature. Certain rooms are also provided with air-extraction systems.

The laboratory is concerned with chemical and physical control of supplies, technical control of works' processes and the investigation of many day-to-day problems which arise in the course of operation and maintenance of London Transport's services and where the specialized facilities available are likely to be of help. This work is essentially of a short-term character, but longer-term investigations are also undertaken, and special mention may be made of the pioneer work relating to the application of low-viscosity oil for lubrication of bus engines and improved lubricants for worm-driven axles; much work has also been done in connexion with lead–acid accumulators, in regard to fatigue failures of railway car axles, and on other problems relating to railway rolling stock and the permanent way.

The facilities for metallurgical work include a metals-chemistry laboratory, a laboratory for mechanical testing, a specially screened room with barium block lining for radiography, also rooms for macro- and micro-examination of metals and for technical photography. Equipment for heat treatment and welding tests is situated in the pilot plant shop. A well-equipped machine shop provides a service for all sections of the laboratory.

The physics laboratories include rooms for testing of batteries, photometric testing of electric lamps, non-destructive testing, high-voltage tests and construction and calibration of instruments. A cold chamber, 14 ft. × 9 ft. × 8 ft. high, is available for tests on equipment at temperatures down to about 0° F.; it can also be adapted for tests at elevated temperatures up to 120° F.

The chemical division of the Laboratory comprises three sections dealing respectively with fuels and lubricants, paints and protective processes and general chemistry, the latter being concerned with all chemical work other than that falling to the specialized sections. Separate 'general' and 'research' laboratories are provided for these various functions. In addition to its normal equipment, the fuels and lubricants section is equipped with an automatic oil oxidation apparatus, equipment for mechanical testing of lubricants and a small engine for determining the ignition quality of road-vehicle fuel. Equipment for testing paints includes a spray booth, stoving oven, an accelerated weathering machine and other apparatus for accelerated tests. There are also various instruments for physical testing, and a special room with direct access to the outdoor exposure station is provided for application tests, on which great emphasis is placed.

A textiles sub-section comprises a room for chemical and general examination of textiles and an air-conditioned room for physical tests, the equipment of which includes a tensile testing machine with autographic recorder, a horizontal fabric strength tester and apparatus for abrasion tests, flex resistance and bursting strength.

The largest item of equipment at present installed in the laboratory is a fatigue testing machine which has been specifically designed to investigate the fatigue strength of railway car axles (Fig. 2). The machine has been developed at the University of Nottingham to determine the strength of specimens of axle steel when subjected to a rotation bending stress. There is a 'scale effect' of uncertain magnitude in fatigue and the machine is therefore designed to test specimens of typical axle sizes, namely, 6½ in. diameter. It is the intention initially to investigate the effects on fatigue strength of surface rolling and, later, the effect of shrink fits. The influence of composition of the steel and heat treatment will also be investigated.

The machine is of the resonant type, with a mechanical drive via a slipping clutch to permit starting and stopping. The specimen is about 5 ft. long × 6½ in. diameter with enlarged ends. Each end is secured in a heavy disk. The assembly of two heavy disks and the specimen as a spring has a natural frequency for transverse bending of the specimen just below 50 c./s. The assembly is freely suspended from the centre of one disk by a wire rope with the specimen vertical. The lower disk carries below it a rigid extension tube. If the lower end of this tube is made to move in a circular path at a speed corresponding to the natural frequency, the assembly is caused to vibrate and so give a rotating bending stress on the specimen. The stress is measured by resistance strain gauges, calibrated in position, and a number of protective devices are included so that the machine may be left running unattended. Two specimens have already been tested in the machine. One failed at a stress not much greater than the expected fatigue limit (2,910,000 cycles at 16 tons/in.^{2}). The other failed at a higher stress (640,000 cycles at 20 tons/in.^{2}). Both specimens were afterwards broken open by static bending to expose the fatigue-fractured surfaces.

The laboratory when up to strength will have a total staff of about eighty of all grades, one-third of whom will be graduates or otherwise professionally qualified.

TEXTILE RESEARCH INSTITUTE OF THE BOMBAY TEXTILE RESEARCH ASSOCIATION

By Dr. C. NANJUNDAYYA
Joint Director

THE cotton textile industry is one of the foremost organized industries in India and it has been playing its many-sided part making an increasingly valuable contribution to the country's economy and progress.

Although the need for organizing research on a co-operative basis was well recognized by the textile manufacturers of India long ago, the financial resources of the industry were not sufficiently encouraging to undertake any scheme in this behalf. It was only during and after the Second World War that impetus was given by the Government of India to promote and organize textile research. In order to implement this, the Government of India promulgated the Cotton Textiles Fund Ordinance in 1944, and constituted the Cotton Textiles Fund Committee for administering the funds raised by the levy of customs duty on yarn and cloth exported from India. This Committee, after due deliberations, decided to establish four textile research institutes, one in each of the four zones, into which the country was divided, and mills situated in Zone I, comprising the old Bombay State to the south of River Tapti, Madhya Pradesh and Hyderabad, were allotted to the jurisdiction of the Bombay Textile Research Association. It was further decided that, in the first instance, two research institutes, one at Bombay and another at Coimbatore, be established, ATIRA at Ahmedabad having already come into existence.

The Millowners' Association, Bombay, then took steps to formulate a scheme for establishing a Textile Research Institute at Bombay. Although the Bombay Textile Research Association was registered in 1954 under the Societies Registration Act, 1860, the preliminaries connected with the acquisition of a suitable plot of land, planning and other formalities connected therewith took some time. The actual construction of laboratory premises was commenced in 1958 on a 23-acre plot of land situated in very pleasant surroundings at Ghatkopar, a suburb of Bombay.

As the construction of the buildings and installation of the pilot plant and laboratory equipment were expected to take two or three years, at the instance of Dr. C. Nanjundayya, joint director, a building was taken on rent in 1958 and such technical work as could be carried out in the mills, namely, standards engineering, statistical quality control, technical liaison, physical and chemical testing, T.W.I., and dissemination of current technical literature, was commenced. As a result of the work done so far, the mills have begun to realize the benefits accruing to them from the Research Association.

The original scheme of the Textile Research Institute envisages a capital expenditure of about Rs. one crore (£750,000) and recurring expenditure of about Rs. 12 lakhs per annum (£90,000).

The scope of work of this Institute covers the entire field of textile research, comprising fundamental and applied research in the mechanical and chemical processing of cotton as well as man-made fibres, operational research, including statistical quality control and human relations, technical liaison for communication of results obtained in the laboratories to the mills and of mill problems to the Bombay Textile Research Association laboratories, an up-to-date technical library and dissemination of technical knowledge to mill technicians, and research in textile engineering.

The present built-up area of the buildings is about 100,000 sq. ft., which is approximately two-thirds of the area that is proposed to be built according to the original scheme.

It was on December 9 that the President of India, Dr. Rajendra Prasad, inaugurated the Textile Research Institute of the Bombay Textile Research Association. Dr. Rajendra Prasad was welcomed by Mr. Krishnaraj M. D. Thackersey, chairman of the Association; in doing so, he stressed the importance of research to the textile industry and said that it could profitably be carried out by co-operative organizations like the Bombay Textile Research Association, which has thirty-six large cotton mill companies as members. He further said that it was the desire and determination of the Research Association to see that the research and development work carried out in this Institute would materially help the textile industry not only to maintain its present level of prosperity but also to enable it to achieve further progress and prosperity.

Dr. Rajendra Prasad, in his opening address, appreciated the co-operative effort of the textile mills in the establishment and maintenance of the Research Institute for their common benefit, and said that it would supplement the general policy of the Government of India to encourage and undertake research in all fields. He was happy to know that the Association had the support of a large number of mills, and he had no doubt that the research done by the Research Institute would benefit the manufacturers, the consumers and the country at large.

Mr. Ramnath A. Podar, deputy-chairman, proposed a vote of thanks to the President, and Dr. C. Nanjundayya read a few of the large number of messages of good wishes received on the occasion from distinguished persons and institutions in India and abroad.

SECONDARY EDUCATION IN INDIA

IN 1952 the Government of India appointed a Commission (the Mudaliar Commission) to examine the prevailing system of secondary education in the country and to suggest measures for its reorganization and improvement. It is about six years since the report of the Commission and its recommendations have been before the public. These are now being considered for implementation by the State Governments under guidance and financial assistance from the centre (*Sci. and Culture*, 26, No. 4, 1960).

According to the new scheme for an eleven-years school course, education in secondary schools extends over 6–7 years. It is to be regarded as a complete unit by itself, and not merely a preparatory stage for the university and technical education.

This pattern of education has been framed generally after that of British countries, but it also includes some features of the American and Soviet systems.

The British pattern differs from that of the United States by providing for specialization at the secondary stage. In the United States, the secondary schools cater for a purely general, or academic, education as a preparatory step for admission to colleges in the university, and/or a vocational course as a preparation for earning a livelihood. The schools in the British countries provide both for those going on to universities and for those going directly into industries and commerce, or the public service.

The Commission has also borrowed some special features from the Soviet system of secondary education. This relates to the introduction of training for some crafts and fine arts in the curriculum so that instruction might be combined with some productive work.

The secondary school curriculum in India, now recommended, is a diversified one. A certain number of core subjects are, however, common to all students, irrespective of the diversified course of study taken by them. These are: (1) languages, (2) general science, (3) social studies, and (4) a craft. The diversified course of study consists of seven groups, namely (1) humanities, (2) sciences, (3) technical studies, (4) commercial subjects, (5) agricultural subjects, (6) fine arts, and (7) home sciences. Three subjects from any one of these groups are to be taken along with the core subjects.

A study of two languages has been recommended for the high and higher secondary stages. One of these must be the mother tongue or the regional language which forms the medium of instruction throughout the secondary stage. English and Hindi have been recommended for introduction in the earlier or lower secondary stage. English and Hindi have to be studied as compulsory languages by all, besides the regional language or the mother tongue.

The introduction of the higher secondary course has given rise to many serious problems, owing to the dearth of competent science teachers, the lack of proper laboratory facilities and equipment, and the shortage of suitable text-books in science subjects with precise descriptions of terms and terminologies for the regional languages.

A sudden increase in the school population at the end of the secondary stage, likely to result from the introduction of the eleven years school course, is another problem that has to be faced. Many of those coming out of the secondary schools will fail to find admittance to the university and professional colleges or the polytechnics; nor are they likely to be absorbed in sufficient numbers in government services or industries. The result will be an increase in unemployment. Though India is generally backward in education and has a literacy of only 17·5 per cent of the population as a whole, its relative position at the secondary level is abnormal in comparison with that of the most advanced countries of the world.

For every thousand students in the primary schools the number at the secondary and university stages for different countries are indicated in Table 1.

The structure of secondary education on India with a literacy of about 18 per cent of the population as a whole rests on an abnormally shrunken and narrow base. This represents instability, and is responsible for the comparatively poor standard of efficiency of the educational system. The remedy lies in the introduction of free and compulsory primary education as early as possible.

Secondary education for girls also represents a serious problem in India. The Mudaliar Commission is not in favour of making any distinction between education imparted to boys and girls in the secondary

Table 1

	No. of students per thousand		
	Primary stage	Secondary stage	University stage
India, 1956	60	23	1·8
China, 1955	87	7 4	0 5
Japan, 1956	144	90	6 8
West Germany, 1956	97	72	2 5
France, 1954	114	30	3·5
United Kingdom, 1954	108	49	1 6
U.S.S.R., 1958	125	38	10 5
United States, 1955	154	48	0 16

	No. of students	
	Secondary stage	University stage
India, 1956	364	30
China, 1955	84	5 6
Japan, 1956	628	47
West Germany, 1956	740	26
France, 1954	264	31
United Kingdom, 1954	463	15
U.S.S.R., 1958	300	84
United States, 1955	313	105

schools, but recommends special facilities for the study of home science for the latter. Separate schools for girls have been considered preferable at the secondary stage, and definite conditions have been laid down for mixed co-educational schools to satisfy the special requirements of girl students and women teachers.

The Secondary Education Commission has considered defects in the present system of teaching and examinations, and has recommended certain changes. These include: reduction in the number of external or public examinations, change in the type of questions giving preference to the objective type of tests over the subjective or essay type, maintenance of a proper record of school work for every pupil, credit for the results of internal examinations and for school records in the final assessments by public examination, provision for only one public examination at the completion of the secondary course, and for compartmental examinations in case of failures in one or more subjects.

Even the organization of a single State-wide public examination at the completion of the secondary course each year is a formidable task as the total number of candidates in any State in India may easily reach a very high figure.

THE BRITISH COUNCIL

THE feature article accompanying the general survey of the year in the British Council's annual report 1959–60 deals with the printed word*. In doing so it gives a most readable account of the work of the Council's libraries and the way in which they foster the demand for British books and periodicals abroad. It includes details of the Council's own publications, and the information work, book exhibitions and book reviews which are incidental or ancillary to these activities. A brief summary of the action taken by the Government to promote exports of British books and periodicals is included. Of some 7,000 periodicals published in the United Kingdom, the Council uses, with yearbooks, some 1,750 in its work abroad and maintains more than 32,000 subscriptions not only for its own libraries but also for presentations, on local advice, to a wide range of schools and colleges, research stations and societies.

The general survey of the year notes that the Council is undertaking the general reception, accommodation, finance and welfare of scholars coming to the United Kingdom under the Commonwealth Scholarship and Fellowship Plan, as well as a corresponding responsibility for teachers from the Commonwealth coming for courses at teachers' training colleges. It has also been asked to help with the recruitment of teachers for schools in Commonwealth countries and with recruitment to university posts. Among developments overseas during the year, those in India and Pakistan are particularly stressed; by the end of 1960–61 the Council will have libraries in eight towns in Pakistan, six of them being university towns, while the tour by a team of eminent representatives of science and medicine in Cairo and the United Arab Republic is likely to increase the exchange of academic and professional visits between the United Kingdom and the Republic. In February 1960 the Council resumed work in Cairo. The programme of exchanges in the educational, cultural and scientific fields drawn up in March 1959 in Moscow with the U.S.S.R. was for the most part carried out, and for the first time British postgraduate students have studied for a full academic year in the Universities of Moscow and Leningrad while Soviet students have studied in British universities.

Between July 1957 and March 1960 the Council directly recruited 253 teachers of English and other subjects in English for overseas, and awarded 280 scholarships for training teachers for overseas in the United Kingdom. The continuing need for this work is again stressed in the report, and summer schools for teachers of English arranged during the year in the United Kingdom were attended by more than 700 teachers from overseas, while summer schools arranged overseas were attended by some 2,000 teachers. Overseas visitors under the auspices of the Council number 6,040, of whom 1,231 were from the Commonwealth and 381 from the Colonies; of these 1,052 were in medicine, 1,359 in science and technology, 1,125 in social science and 1,589 in education. A higher proportion is now coming from the Indian sub-continent and the Far East, and a smaller proportion from Europe. The programme of study tours continues to expand, and, of the 65 such tours arranged during the year, 15 were in science and technology. Stress is also laid on the value of the many specialist tours and advising visits overseas by leading figures in many professions which the Council arranges, and in regard to science the report refers to the continued financing of the exchange of senior scientists and research workers between the Royal Society and the U.S.S.R. Academy of Sciences, while the Royal Society was also enabled to invite a delegation from the Roumanian Academy of Sciences to visit Britain. The Council's current policy on books and periodicals places greater emphasis on scientific, technical and medical text-books.

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* The British Council. Annual Report, 1959–1960. Pp. vi+110+12 plates. (London: British Council, 1960).

The total number of overseas students in the United Kingdom is estimated at about 47,000, and during the year the Council met on arrival 8,137 students as well as 1,299 United Nations' Fellows, Colombo Plan trainees, etc., who were assisted to find accommodation. Permanent accommodation in lodgings was found for 3,417 students, and for 405 in Council residences, and temporary accommodation for 5,005 while on study visits, etc., apart from transit or temporary accommodation in lodgings or Council residences for many more. Some 9,878 students enrolled as members of Council Centres during 1959, 2,736 attended vacation courses of 7–15 days, 1,481 week-end courses, and 9,554 half-day or full-day study visits to factories, museums, etc.

A SCIENCE PLANNING BOARD

THE proposal that the Government should set up a Scientific and Technical Planning Board to give "better direction to our scientific effort" is one of the recommendations for immediate action by the Association of Scientific Workers in a policy statement, "Science and Education"*.

The statement declares that, "given an adequate structure there is no reason whatever to fear a shortage of suitable people for higher education in science and engineering and teaching. Considerable untapped resources exist". The number of women in British technical colleges is very small compared with men. This difference is by no means due to innate intelligence or leanings, but largely to social customs reflected in school policies exacerbated by the desperate shortage of science teachers in girls' schools.

The Association's statement lists five objectives: to make science a central subject throughout the school lives of all children; to expand considerably the provision of educational opportunities for children beyond the age of fifteen; to overcome the critical shortage of science and mathematics teachers; to achieve a larger annual output of scientists and engineers than the present Government's targets, as soon as possible; to raise considerably the annual output of technicians of all kinds.

The report states that the first and most fundamental need is to provide more science teachers in schools and in technical colleges, training colleges and universities—and the most acute need is in schools. The only permanent solution to a shortage is to raise the salaries of teachers. The Association believes that "the immediate critical shortage" could be alleviated by a government-led national programme of secondment or release of scientists and mathematicians from the Civil Service (including the defence departments), nationalized industries and universities to teach in schools while the 'bulge' passes through, and a national drive to bring more married women back into teaching.

Proposals are also made to have at least four, and preferably six, new university institutions before the end of the 1960's; to expand the work of the technical colleges; to improve conditions of service of technicians in higher educational establishments; to assist more students to train to be scientists; and to make the fullest possible use of trained scientists, technologists and technicians.

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* Association of Scientific Workers. Science and Education: a Policy Statement by the Association of Scientific Workers. Pp. 16. (London: Association of Scientific Workers, 1960.) 1s.

TOBACCO SMOKE RESEARCH

THE Tobacco Manufacturers' Standing Committee has as a declared aim the assistance of research into questions concerned with the relationship between smoking and health. That this object is being fulfilled is evident from its report for the year ended May 31, 1960, which summarizes investigations carried out during the year under the auspices of the Committee or with its financial support, including research into factors affecting smoking habits, the biological activity of tobacco smoke, factors affecting the incidence of lung cancer and certain other diseases and physiological and psychological aspects of smoking*. In addition to supporting these activities, the Committee has also made financial grants to the Medical Research Council and to the Institute of Cancer Research, Royal Cancer Hospital, London, for research into causes of lung cancer and into tumour viruses.

Fractions of cigarette smoke condensate prepared in the laboratories of the Committee have been found by several workers to have carcinogenic or tumour-promoting properties, but as the report points out, these results, obtained by application of smoke fractions to animal tissues, are not necessarily reliable guides to the possible response of human lung tissue to tobacco smoke.

Some of the investigations supported by the Committee have been stimulated by the suggestion of Sir Ronald Fisher that smoking habits may be influenced by genetic factors. It has been found in one investigation that the smoking habits of identical twins are more alike than those of non-identical twins, and other work into the personality characteristics of different types of smokers appears to confirm the view that genotypic differences exist between smokers and non-smokers and between cigarette smokers and pipe smokers.

The Committee has also published the first Supplement to its third research paper, "The Constituents of Tobacco Smoke: an Annotated Bibliography" (which is edited by H. R. Bentley and E. G. N. Berry)†. This useful compilation provides a convenient list of references, arranged in sections corresponding to chemical classes, covering the literature which appeared during 1959, and will be an invaluable guide to research workers in this field.

W. CARRUTHERS

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* Tobacco Manufacturers' Standing Committee. Report for the Year ended 31st May, 1960. Pp. 11. (London: Tobacco Manufacturers' Standing Committee, 1960.)

† Tobacco Manufacturers' Standing Committee. Research Papers, No. 3: The Constituents of Tobacco Smoke, an Annotated Bibliography. First Supplement. Edited by H. R. Bentley and E. G. N. Berry. Pp. 1+25. (London: Tobacco Manufacturers' Standing Committee, 1960.)

THE WOLFSON FOUNDATION

THE first report of the Wolfson Foundation, created in 1955, covers the first five years work of the Foundation*. So far, the Foundation has adopted as its principal aims the advancement of education and health in the United Kingdom and Commonwealth, with particular reference to scientific and technological education, building for higher education, youth activities, medical research and education, cancer research, and medical, surgical and nursing services not covered by the National Health Service. Up to July 1958 it was occupied by preliminary work, including the creation of the necessary administrative organization and a Board of Trustees of wide experience and enjoying general confidence. To November 30, 1960, grants totalling £3,311,110 had been made and grants totalling a further £237,500 were made in December 1960. These latter grants, which are listed on a separate sheet issued with the report, include £100,000 to St. Hilda's College, Oxford, for a residential block for 60 undergraduates, £52,000 in extension for three years of the £125,000 already granted to the National Playing Fields Association, and £5,000 to the Commonwealth Society for the Deaf for a rehabilitation centre in Eastern Nigeria.

Grants for education noted in the report include £250,000 for a new Commonwealth Hall of Residence for 400 men students at the University of London; £65,000 to endow the Wolfson chair of metallurgy at the University of Oxford; and £4,500 for a research fellowship in metallurgy there; £300,000 for a hall of residence for about 150 men students at the University of Glasgow; £70,000 for an assembly hall forming part of the main block of Churchill College, University of Cambridge, £150,000 for the establishment of the Institute of Criminology in the University of Cambridge; £75,000 for building and furnishing the library of St. Catharine's College, Oxford; £350,000 to the Imperial College of Science and Technology to build and equip laboratories for biochemistry and chemical microbiology and support teaching and research in these subjects; £210,000 to the Royal Society to endow a research professorship; £100,000 to New Hall, Cambridge, and £80,000 to St. Anne's College, Oxford. A grant of £2,000 to the Royal Institution is a contribution to the Institution's work in interpreting current scientific developments to the public.

Major grants in the field of health include £250,000 to the Westminster Hospital School of Nursing; £150,000 over 10 years to the British Empire Cancer Campaign in support of its researches; £150,000 to the Postgraduate Medical School, Hammersmith, for the Wolfson Institute containing lecture theatres, common rooms and other amenities for students and teaching staff; £184,000 for the Institute of Psychiatry associated with Maudsley Hospital, University of London; and £450,000 to the Royal College of Physicians for a new building containing a larger library, a main lecture theatre seating 300, a smaller lecture theatre, committee and common rooms, and a dining hall. A grant of £22,000 was made to Mr. Wylie McKissock, neurological surgeon at St. George's Hospital, London, for surgical research into anatomical and biochemical aspects of hæmorrhage of the brain.

A grant of £10,000 to the Royal Geographical Society is to be used during 1960–63 to encourage participation of young people in exploration. Miscellaneous grants include £2,500 to the Crofters' Commission, £4,631 to the British Museum to acquire a sculpture, discovered at Nineveh, showing Babylonian prisoners being carried off by the Assyrians, and £2,500 to the Justice Educational and Research Trust for an inquiry into the investigation of complaints against administrative and executive decisions.

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* Report of the Wolfson Foundation, 1955–1960. Pp. 39 (8 plates). (London: The Wolfson Foundation, 1960.)

PUBLICATION OF CALORIMETRIC AND THERMODYNAMIC DATA

THE "Resolution on Publication of Calorimetric and Thermodynamic Data", which was adopted by the fifteenth Calorimetry Conference held at Gatlinburg, Tennessee, during September 7–10, 1960, is a revised and extended form of the similar resolution adopted by the eighth Calorimetry Conference held in 1953. It outlines minimum publication standards for the guidance of authors, editors and referees of articles on calorimetry, and states the author's first responsibility is to report his results in a form related as closely as possible to the experimentally observed quantities, with enough details of the experiments and auxiliary information to characterize the results completely. Derived or secondary data should never be published without the primary data on which they are based. The auxiliary information should include a complete description of the apparatus and procedures, with details of the reaction vessel or calorimeter, the temperature and time measuring systems, the calibration and sensitivity of the instrument used and the precision and accuracy of the results obtained; the source and method of preparation of the samples studied, together with analyses and criteria of purity; and the values of the units and physical constants used in the calculations.

One section of the resolution deals in some detail with the presentation of results, and gives recommendations for the thermochemical data, thermal data, and calculated thermodynamic functions which should be included in the description of certain important kinds of thermodynamic studies. It is pointed out that graphical or analytical representation of results is sometimes worth while for the convenience of the reader, but such representations seldom are a satisfactory substitute for tabular presentation of accurate experimental results. Where rough measurements made for technical purposes, or measurements on materials of undefined composition or by inferior techniques, are reported, they should be given minimum journal space and the presentation should clearly recognize the lack of reliability of the results.

PROTEIN AND RIBONUCLEIC ACID IN THE GROWTH OF FERN GAMETOPHYTES

IN many Polypodiaceae, the germinating spore yields a filamentous protonema and rhizoid cell, that is, a 'one-dimensional' growth. After a certain time-lapse, the growing protonema begins to divide by longitudinal walls also, thereby yielding a plate-like structure, or 'two-dimensional' system. This leads on to the typical heart-shaped prothallus or gametophyte. Y. Hotta (*Jap. J. Bot.*, 17, 214; 1960) has now contributed new facts about the role of protein and ribonucleic acid in the differentiation of the gametophyte of *Dryopteris erythrosora*.

Estimation of the protein contents during the course of development clearly indicates that whenever the two-dimensional growth takes place, a sharp increase in protein concentration in the cell can be observed, whereas in the course of the primary one-dimensional growth, protein concentration is gradually lowered. The two-dimensional growth can be specifically inhibited by some amino-acid analogues such as ethionine and 5-methyltryptophan, and is converted to the 'artificial' one-dimensional growth.

The same selective interference of the two-dimensional growth is also brought about by 8-azaguanine, the effective inhibitor of the nucleic acid synthesis. This inhibition can be alleviated by adding corresponding amino-acids or guanine, respectively. Regardless of the nature of the effective inhibitors used, the selective inhibition is always accompanied by an immediate cessation of increase in the protein concentration followed by its gradual decrease. The author has also shown that ribonucleic acid from the two-dimensional prothallium possesses a nucleotide composition distinct from that in the one-dimensional growth. Ribonucleic acid found in the latter, induced artificially by 8-azaguanine, has been shown to have practically the same nucleotide composition as that of the primary one-dimensional growth. Accordingly, it has been tentatively inferred that a special ribonucleic acid fraction which is sensitive to the action of 8-azaguanine is closely related to the two-dimensional growth.

VIBRATIONALLY EXCITED NITRIC OXIDE PRODUCED IN THE FLASH PHOTOLYSIS OF NITROSYL CHLORIDE

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WHENEVER a molecule dissociates by absorbing radiation of shorter wave-length than is required from the known strength of the bond broken, the problem arises of the distribution of the excess energy among the fragments. Frequently it can be shown that one or more of these fragments are produced in an electronically excited state, but, in general, there is some energy which must appear in other forms. For a diatomic molecule, it follows that the atoms are produced with excess kinetic energy, but where a polyatomic molecule is concerned, there is the possibility that one or more of the products are produced vibrationally or rotationally excited in the ground electronic state. The present communication describes what seems to be the only direct experimental evidence that these possibilities are, in fact, realized.

In the flash photolysis of sulphur trioxide, Norrish and Oldershaw[1] observed the presence of vibrationally excited SO with $v'' \leqslant 3$, but, though it was shown that the effect was not thermal, it was not possible to decide whether the excited molecule was produced by primary photodissociation or by secondary reactions, that is:

$$SO_3 + h\nu \rightarrow SO^* + O_2 \qquad (1)$$

or

$$SO_3 + h\nu \rightarrow SO_2 + O(^1D) \qquad (2a)$$

followed by

$$O(^1D) + SO_2 \rightarrow SO^* + O_2 \qquad (2b)$$

Similarly, in the flash photolysis of nitrogen dioxide, Basco and Norrish[2] observed the first vibrationally excited level of nitric oxide, but again it was not possible to decide whether it is a primary or secondary product, in the latter case derived from:

$$O + NO_2 \rightarrow NO^* + O_2 \qquad (3)$$

We have now studied the flash photolysis of nitrosyl chloride in the presence of a great excess of inert gas to ensure isothermal conditions and have demonstrated the presence of very highly vibrationally excited nitric oxide. Many absorption bands in the region 2100–4000 Å. have been observed and it has been possible to assign most of them to transitions from the ground state of NO ($X^2\Pi$) to the $A^2\Sigma$, $B^2\Pi$, $C^2\Pi$ and $D^2\Sigma$ excited states and thus to demonstrate that all vibrational levels of the ground state up to $v'' = 10$ are populated. Some bands remain unassigned or only tentatively so, but it seems likely that these also will be found to correspond to transitions from vibrationally excited levels of the ground electronic state of NO. The decay of the excited molecules is very rapid and those in the higher vibrational levels are only present in appreciable concentrations during the photolytic flash.

Nitrosyl chloride can be decomposed by light of wave-length below about 6400 Å.[3] and, even at this wave-length, there is a slight excess of energy over that required to break the ON—Cl bond. Its extinction coefficient in the ultra-violet below 3350 Å. increases towards a maximum at around 1970 Å.[4] and, under the conditions obtaining in the experiments reported here, most of the absorption occurs in the region below approximately 2700 Å. Assuming the transmission of the quartz flash-lamp and the reaction vessel is negligible below about 1950 Å. the excess energy available lies between about 70 kcal. and 110 kcal. It is now evident from our spectra that at least 50 kcal. of this excess energy can appear as vibrational energy of the nitric oxide molecule and we suggest that it is in the primary process :

$$\mathrm{NOCl} + h\nu \rightarrow \mathrm{NO}^* + \mathrm{Cl} \qquad (4)$$

that this occurs.

The possibility that the NO is initially produced in an electronically excited state from which it enters vibrationally excited levels of the ground state has also been considered. This seems unlikely as there is no known electronic state of NO which, on spin conservation and energetic grounds, could qualify. Experiments designed to set an upper energy limit for this hypothetical electronic state are in progress, with particular reference to the $^4\Pi$ state predicted by Mulliken[5] to lie 4·5 eV. above the ground state.

It is also theoretically possible that NO produced initially without excitation could be excited by the flash. In the flash photolysis of NO this excitation has been realized[6], but it was shown that the reaction :

$$\mathrm{NO}\,^2\Pi(v = 0) + h\nu \rightarrow \mathrm{NO}\,^2\Sigma(v = 0, 1) \qquad (5)$$

followed by

$$\mathrm{NO}\,^2\Sigma(v = 0, 1) + M \rightarrow \mathrm{NO}\,^2\Pi(v = 0, 1, 2 \ldots) \qquad (6a)$$

or

$$\mathrm{NO}\,^2\Sigma(v = 0, 1) \rightarrow \mathrm{NO}\,^2\Pi(v = 0, 1, 2 \ldots) + h\nu \qquad (6b)$$

only produces an appreciable concentration of the first excited level, with the second only just detectable under favourable conditions. It follows that this mechanism can be excluded.

Two other reactions, namely :

$$\mathrm{NOCl} + \mathrm{Cl} \rightarrow \mathrm{NO}^* + \mathrm{Cl}_2 \qquad (7)$$

and

$$2\mathrm{Cl} + \mathrm{NO} \rightarrow \mathrm{NO}^* + \mathrm{Cl}_2 \qquad (8)$$

can be excluded by virtue of the fact that, when chlorine was photolysed in the presence of NOCl using a 'Pyrex' glass filter virtually to exclude the direct photolysis of NOCl, NO* was not observed. For the higher levels, the former can also be excluded on energetic grounds.

There is convincing evidence[1,7,8] that in exothermic reactions of the type :

$$A + BCD \rightarrow AB + CD \qquad (9)$$

up to 100 per cent of the energy liberated can appear as vibrational energy of the newly formed molecule *AB*. Reactions (2*b*) and (3) have provided the only indication so far that the molecule *CD* may be vibrationally excited to some extent ; but the fact that reaction (7) does not produce a detectable concentration of NO* militates against this. As there is no evidence for the presence of Cl_2^* here or for O_2^* being produced in reaction (2*b*), the question remains open.

If it be admitted that the primary process is :

$$\mathrm{NOCl} + h\nu \rightarrow \mathrm{NOCl}^* \qquad (10)$$

rather than direct photodissociation, two other possibilities exist, namely :

$$\mathrm{NOCl}^* + \mathrm{NOCl} \rightarrow \mathrm{NO}^* + \mathrm{NO} + \mathrm{Cl}_2 \qquad (11)$$

and

$$\mathrm{NOCl}^* + \mathrm{NO} \rightarrow \mathrm{NO}^* + \mathrm{NOCl} \qquad (12)$$

Reaction (10) was proposed by Kistiakowsky[3] for decomposition at longer wave-lengths on the grounds that the absorption spectrum showed bands with line structure and that decomposition of NOCl could be achieved by light below 6400 Å., whereas the heat of dissociation (taken as 46·4 kcal.) corresponded to light below 6130 Å. The latter reason is invalid if the value of 37–38 kcal. is taken for the NO—Cl bond[9,10] and, as both he and Allmand[11] pointed out, the fact that the quantum yield of 2 for photolytic decomposition of NOCl is not reduced by a seven-fold dilution with nitrogen or a nine-fold reduction in pressure indicates primary dissociation. At the shorter wave-lengths this would appear to be even more likely. In the present work the excess of nitrogen as coolant over nitrosyl chloride and the pressure of nitrosyl chloride itself have each been varied by a factor of more than 100 without producing any fundamental change in the results obtained. It has also been shown that addition of small quantities of NO to NOCl produces no major effects. Larger quantities decrease the population of the higher levels, though they seem to increase that of the lowest excited level probably because of the reactions :

$$\mathrm{NO}(v = n) + \mathrm{NO}(v = 0) \rightarrow \mathrm{NO}(v = n - 1) + \mathrm{NO}(v = 1) \qquad (13)$$

To summarize, it appears likely that only reaction (4) could reasonably be expected to produce vibrationally excited NO in the yields obtained. The question arises as to the generality of this reaction ; but at least it can be said that the present evidence lends plausibility to reaction (1) and the corresponding reaction with NO_2. In the production of O_2^* by the flash photolysis of ozone[12], the evidence for the mechanism :

$$\mathrm{O}_3 + h\nu \rightarrow \mathrm{O}_2 + \mathrm{O}(^1D) \qquad (14)$$

and

$$\mathrm{O}(^1D) + \mathrm{O}_3 \rightarrow \mathrm{O}_2^* + \mathrm{O}_2 \qquad (15)$$

is convincing, though the possibility of a contribution from the reaction :

$$\mathrm{O}_3 + h\nu \rightarrow \mathrm{O}_2^* + \mathrm{O} \qquad (16)$$

cannot now be excluded.

In preliminary experiments it was found that mixtures of nitric oxide and bromine, on standing for a short while, develop a continuous absorption spectrum in the ultra-violet which may reasonably be attributed to NOBr. Results generally similar to those with NOCl were obtained on flashing this mixture.

One of us (N. B.) thanks Imperial Chemical Industries, Ltd., for the award of a fellowship.

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IN VITRO TRANSFORMATION OF NORMAL CELLS BY POLYOMA VIRUS

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THE polyoma virus[1] provides favourable material for investigating the relationship between the lytic and the non-lytic interaction of a virus with animal cells, and for studying the cellular transformation which results in the development of tumours. A necessary requirement for such studies is the establishment of *in vitro* systems of analysis. It has been shown, in an *in vitro* system, that the lytic interaction of polyoma resembles that of some other viruses that do not produce tumours[2]; and it was further found that after *in vitro* infection of normal mouse embryo cultures it is possible to establish a line of cells with a stable cell-virus association[3]. The present experiments were undertaken in order to determine the consequences of infection of mouse kidney cells, and to develop for rat and hamster cells an *in vitro* system for the non-lytic transformation that results in the development of tumours. Rat and hamster tumours are rapidly produced by polyoma *in vivo*[4], and *in vitro* infection of normal embryo and kidney cells of these species showed no obvious cytopathic effect under conditions that produced a clear cytopathic effect on normal mouse cells.

Kidneys were used for the mouse experiments, since cells from this organ are very susceptible to tumour induction *in vivo*. In the first experiments, cultures were made of trypsin-dispersed kidney cells from 12-day-old mice that had been inoculated subcutaneously with polyoma virus *in vivo* when 2 days old. The cultures were seeded with 2×10^6 cells in 50-mm. Petri dishes with 4 ml. of medium consisting of 0·5 per cent lactalbumin hydrolysate in Earle's saline (*LA*-medium) and 10 per cent horse serum. The entire medium was charged every 2 or 3 days. These cultures formed monolayers, but at 5–6 days after seeding there was a strong cytopathic effect, and there was generally at this time virus with a hæmagglutination titre of 8,092 per ml. in the culture fluid. It was also found at this time that, in addition to the cytopathic effect, there were groups of proliferating fusiform cells that did not undergo lysis despite the high virus titre in the medium. These cells were therefore presumably resistant to lysis by superinfection, in the same way as cells of the line that had been established from mouse embryo cultures[3].

The proliferating cells in the kidney cultures continued to grow and could form monolayers. But there were always some cells in the population that lysed, so that continued growth was dependent on the balance between cells that lysed and those that continued to divide. There was the same sequence of events in kidney cultures from 4-day-old mice inoculated with virus *in vivo* when 2 days old, and in monolayers of normal kidney cells from 7 to 10-day-old mice infected *in vitro* at a virus to cell ratio of 10 plaque-forming units per cell. The initial number of proliferating cells from mouse kidney cultures infected *in vitro* was much higher than in comparable experiments with mouse embryo cultures. The existence of proliferation and a lytic response in cells from the same organ has also been observed in organ cultures of mouse salivary glands[5].

Proliferation and lysis were found in all the kidney cultures, which were maintained for 1 month, and even in the line of mouse embryo cells[3] there was still some lysis after more than 1 year *in vitro*. The frequency of lysis seemed to depend on nutritional conditions, and in the mouse embryo line there was, after 1 year, no obvious lysis if the medium was charged daily, but a clear cytopathic effect if there was no change of medium for 3 days. It has been postulated that the non-lytic interaction of polyoma may be associated with the establishment of a provirus[6]. The detection of the non-lytic interaction in the mouse may, however, be complicated by the possibility that there may be a cytopathic effect as the result of lysis due to unstable conditions as in bacterial lysogeny[7], and/or to virus induction. The direct evidence for induction of polyoma virus will be discussed elsewhere (Winocour, E., and Sachs, L., unpublished work).

Kidney and embryo cultures were used for the rat and hamster experiments. Although rat cells could be satisfactorily cultured with *LA*-medium and 10 per cent horse serum, with this medium there was an early degeneration of hamster cultures. However, hamster cells could be grown in Eagle's medium with 4-fold concentration of amino-acids and vitamins (*EM*-medium) and horse serum, and grew even better in *EM*-medium and calf serum. 2×10^6 trypsin-dispersed cells from rat and hamster embryos, and kidneys of 7–9-day-old animals, and from hamster kidneys of adult (2–3 months old) animals were seeded in 50-mm. Petri dishes with 4 ml. of culture medium. The cultures were infected after 2–4 days, at a virus to cell ratio of 1 to 2 plaque-forming units per cell. Virus was adsorbed for 1 or 2 hr., and the possibility of different nutritional requirements for transformation was tested by using different media.

Following the assumption that the non-lytic interaction may be associated with the establishment of a provirus, it was further assumed that there might be another analogy to bacterial lysogeny, in that the frequency of the non-lytic transformation might be increased by the use of lower temperatures during the stages leading to the incorporative interaction[7]. Infected cultures with their controls were therefore also kept for various periods, after the initial seeding, growth and virus adsorption at 37° C., in an incubator with a humidified atmosphere and a constant flow of carbon dioxide in air maintained at 24° C. and then transferred again to an incubator maintained at 37° C.

Some representative results of the rat and hamster experiments are given in Table 1. The results show that non-lytic cellular transformations (as described later) can be obtained in rat and hamster after *in vitro* infection with polyoma virus. The transformations were dependent on the cultural conditions

Table 1. CELL TRANSFORMATIONS AND ACIDITY IN RAT AND HAMSTER CULTURES INFECTED WITH POLYOMA VIRUS *in vitro*

Tissue	Exp. No.	Time at 24° C. (days)	Medium	Morphological transformation observed	Acidity
Rat embryo	126†	0	*EM–HOS*	+	+
	126	0	*EM–CAS*	−	+
	117*	0	*LA–HOS* or *CAS*	−	−
	132*	7	*LA–HOS* or *CAS*	+	+
	132	7	*EM–HOS*	−	+
	125	5	*EM–CAS*	−	+
Rat young kidney	123*	0	*EM–HOS*	−	−
	123	0	*EM–CAS*	−	±
	123	7	*EM–HOS*	−	−
	123	7	*LA–HOS*	+	+
Hamster embryo	131*	0	*EM–HOS*	−	±
	131	0	*EM–CAS*	+	+
	131	8	*EM–HOS*	+	+
	130†	8	*EM–CAS*	+	+
Hamster young kidney	127*	0	*EM–CAS*	−	+
	127	7	*EM–HOS*	+	+
	128†	7	*EM–CAS*	+	+
Hamster adult kidney	122*	0	*EM–HOS*	−	−
	122	0	*EM–CAS*	−	+
	122	5	*EM–HOS*	+	+

EM., Eagle's medium with four-fold concentration of amino-acids and vitamins; *LA*, 0·5 per cent lactalbumin hydrolysate in Earle's saline; *HOS*, horse serum; *CAS*, calf serum, 10 per cent serum.

Col. 5: +, transformation; −, no difference observed between infected and control.

Col. 6: +, strong colour difference between infected and control; ±, slight colour difference; −, no colour difference.

* Entire medium changed every 3 or 4 days.

† No change of medium after virus infection.

and the medium used. Thus, for example, with respect to morphological changes, no transformation was observed (exp. 132) in rat embryo cultures with 7 days at 24° C. in *EM*-medium and horse serum, whereas there was a clear transformation under the same cultural conditions with *LA*-medium and horse serum. In the hamster embryos (exp. 131) transformation was observed with calf but not with horse serum, in cultures maintained only at 37° C., whereas transformation with horse serum was found in cultures kept at 24° C. for 8 days after infection. Preliminary experiments on different times at 24° C. indicated that 5–8 days was a suitable time, and transformation was observed in all tissues with the appropriate medium maintained at 24° C. for this period. It also appeared that there was a clearer transformation with a longer time-interval between medium changes. The transformations were observed between 9 and 14 days after infection, and experiments were terminated at 17–26 days.

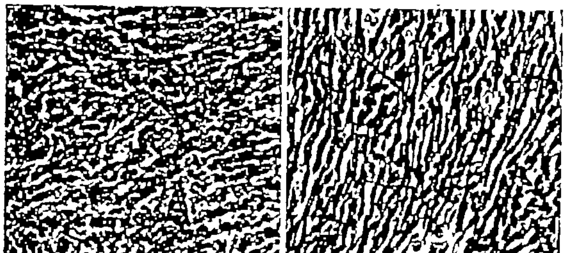

Fig. 1. Control culture of rat embryo cells. Fig. 2. Culture of rat embryo cells with fusiform transformation. Fourteen days after *in vitro* infection with polyoma virus. Methanol fixation, unstained, phase contrast. (× *c*. 112)

In some experiments, in both rat and hamster, there was a regular transformation to fusiform cells (Figs. 1 and 2). In other experiments, with hamsters, fusiform cells tended to form strands with the cells piling up on one another. The difference in appearance of the transformed cultures may have been due to the different conditions under which the cells were grown. The piling-up observed was similar to that produced after *in vitro* infection with Rous sarcoma virus[8]. The transformation by tumour viruses thus presumably includes a loss of contact inhibition[9]. The observation of a transformation is, of course, dependent on a recognizable difference from non-infected control cultures, and in some cases morphological changes may have occurred in infected cells which were not sufficiently different from the controls to be detected readily in the living cultures.

In addition to the morphological changes, there was a difference in the acidity of the medium, in that the transformed cultures were obviously more acid than their controls. There were also some cases (Table 1) of a more acid medium in infected cultures without a morphological difference from the controls. The difference in acidity is presumably due to the changed metabolism of transformed cells. A further difference in both rat and hamster was the better survival of infected cultures under conditions where the controls degenerated. There was also in some experiments a better growth in infected cultures where the controls did not degenerate; and a difference in response to trypsinization. During trypsinization, the cells of controls readily formed cell suspensions, whereas the infected cultures came off the glass either with difficulty and/or in clumps that did not readily disperse.

The above experiments have shown that, in addition to an *in vitro* system for the study of the lytic interaction with polyoma virus, a suitable system is now available for the *in vitro* analysis of the non-lytic interaction associated with cellular transformation. Since the *in vitro* transformations observed include changes in cell morphology, cell metabolism, and what is presumably a loss of contact inhibition, the question arises whether all these changes are required for the malignant transformation. Experiments are therefore now in progress on the growth of the cell cultures in animals.

Note added in proof: Since the above was written, progressive growth has been obtained after inoculation of *in vitro* transformed hamster cells (taken at 14–16 days after *in vitro* infection) into adult hamsters, thus showing that these cells were neoplastic when tested *in vivo*. An independent finding of *in vitro* transformation of hamster embryo cells has also recently been reported[10].

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CARBONIC ANHYDRASE AND THE INNER EAR

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THE finding of carbonic anhydrase in the ciliary body[1] and in the choroid plexus[2] initiated a long series of experiments involving the activity of this enzyme in the secretion of the aqueous humour and cerebrospinal fluid, and also in the treatment of glaucoma and hydrocephalus[3,4]. We were prompted by results from those experiments to search for carbonic anhydrase in the tissues of the inner ear, and to see what effects the specific inhibitor acetazolamide ('Diamox') might have on the composition of the endolymph.

Seventeen cats, initially anæsthetized with sodium pentobarbital (42 mgm./kgm. intraperitoneally), were used. The middle ear was approached from the ventral side, the bulla was removed and samples of perilymph and endolymph were taken using the approach and technique described by Aldred, Hallpike and Ledoux[5]. In twelve experiments, the fluid was collected by means of capillary tubes; the fluid was then put into small test-tubes and 5-λ samples were taken by ultra-microscopic pipettes. In the remaining experiments the capillary tubes were weighed before the samples were taken, again after sampling, and the volumes were calculated from the weights. After the samples had been taken, the tissues were dissected out under × 40 magnification of the microscope. The apical, middle and basal turns of the cochlea, the vestibule (comprising mainly utricle, saccule, ampullæ and the anterior semicircular canal) and the saccus endolymphaticus, which was approached posteriorly, were all removed and analysed for carbonic anhydrase by the method of Maren, Ash and Bailey[6]. The results of these analyses, compared with those from other tissues in the cat[2], are shown in Table 1. These results, taken with earlier estimations in a variety of tissues[7], gave the surprising result that the tissues of the cochlear partition possess the highest known concentrations of carbonic anhydrase activity of any organ assayed.

There appeared to be a specific distribution of enzyme, with the highest concentrations in the apical turn and saccus endolymphaticus, progressively lower concentrations in the middle and basal turns, and lowest concentrations in the vestibule (Table 1). The striking difference between the carbonic anhydrase concentrations of the cochlea and vestibule deserves comment here. The explanation of the differences may lie in the cytological differences found by Smith[8] in the epithelial cells of the stria vascularis and of the vestibule. Pease[9] showed that organs active in the transfer of fluid are characterized by a great increase in the basal cell surface, and that in kidney tubules, submaxillary gland, choroid plexus, and ciliary body, intensive folding of the basal plasma membrane occurs. All these organs have high concentrations of carbonic anhydrase. Similar structural characteristics have been found to be present by Smith in the utricle[10] and in the stria vascularis and spiral prominence[8]. The stria vascularis, however, is a unique structure in the entire membranous labyrinth. On the other hand, it seems certain that endolymph must be formed and absorbed in the vestibule as well as in the cochlea. Smith[8] suggests that transfer of fluid may be more rapid in the stria vascularis, but also that "the cytological specialization of the marginal cells" of the stria vascularis "suggests some additional process and the question arises whether it may not be also the site of some activity specific to the cochlea". This "cytological specialization" consists of beaded vesicles found in abundance in the basal portions of the marginal cells of the stria vascularis. Palade[11] has observed them in macrophages and suggested that they may be a route for pinocytosis and phagocytosis. One cannot help but speculate that this specialization may be concerned in some way with carbonic anhydrase activity, and that the vesicles may represent sites of concentration of the enzyme.

Control electrolyte data were compiled on plasma, cerebrospinal fluid, perilymph and cochlear endolymph (Table 2). In general these conform closely to the data previously reported by others[12–14]. In confirmation of the work of Smith *et al.*[12], and of Citron and Exley[13], we have found high concentrations of potassium in the endolymph, although this is clearly subject to considerable individual variation. Both these groups have also reported wide ranges of potassium concentration in the cochlear endolymph, but have presumed that the lower values must be artefactual in their origin. The data given by Citron and Exley[13] are presented in Table 3 with their reasons for the values of endolymph potassium. They report that it was the Na^+ and K^+ concentrations *per se* of the endolymph in many samples which suggested contamination with perilymph, and only three samples showed the high values given in the table. In our experiments (Table 3) there are numerous possible reasons for this variation: the difficulty of sampling less than 5λ of fluid, the magnitude of volumetric error, and contamination from perilymph, especially in view of the fact that we do not have any histological sections of this material; it may also be that the potassium concentration of the endolymph is subject to considerable variation physiologically, similarly

Table 1

Tissue	Species	Enzyme units/gm. wet tissue
Choroid plexus (ref. 2)	Cat	262
Kidney (ref. 2)	Cat	275
Ciliary process (ref. 1)	Rabbit	30
Whole blood (ref. 2)	Cat	950
Whole blood (present study)	Cat	943
Inner ear (present study)		
Basal turn	Cat	1,086 ± 314
Basal + middle turns	Cat	1,470
Middle turn	Cat	2,263 ± 556
Apex + middle turn	Cat	2,380
Apex	Cat	3,162 ± 1,180
Saccus endolymphaticus	Cat	3,000
Vestibule	Cat	81 ± 17

Concentration of carbonic anhydrase in inner ear and other tissues. Data obtained from six cats. In two cats, it was impossible to separate middle from basal and apical turns. Only two cats yielded data on the saccus endolymphaticus.

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Table 2

	Before acetazolamide				After acetazolamide			
	Plasma	Cerebrospinal fluid	Perilymph	Endolymph	Plasma	Cerebrospinal fluid	Perilymph	Endolymph
CO_2 m*M*/l.	17 2 (9)	21·1 (4)	16·9 (7)	18 6 (5)	11·8 (8)	21 4 (1)	17 6 (2)	—
Cl^- m*M*/l.	120·5 (9)	133 4 (4)	143·4 (6)	157·2 (2)	120 0 (8)	137·1 (1)	158·5 (4)	140·7 (4)
K^+ m*M*/l.	4 3 (6)	4 5 (2)	7·9 (6)	67 (7)	3 6 (8)	3·7 (1)	6 2 (7)	15 (7)

Mean carbon dioxide, chloride and potassium concentrations before and 6–8 hr. after administration of acetazolamide (see text). Numerals in brackets indicate sample number of animals used. For fuller details of potassium concentrations in endolymph see Table 3.

to the potassium concentration of the urine, which is also dependent on a secretory mechanism. It is, however, evident from the data of all workers that potassium is the chief cation of the endolymph, and presumably this unique characteristic reflects the secretory activity of the cells of the cochlear partition. The large variation in K^+ concentration must at present remain unexplained.

Potassium concentration of the perilymph ranged from 6–11 m.equiv. except in one sample, which contained 32 m.equiv.

In six cats, acetazolamide was given intravenously (50 mgm./kgm. as the sodium salt) at zero time and again six hours later, followed by the same dose given intraperitoneally after 12 hr. In some cases pentobarbital anæsthesia was used to facilitate the intravenous injection. 15 hr. following the initial injection of acetazolamide, the dissection procedures described here were carried out. In five experiments, samples were taken from the inner ear of one side in the normal anæsthetized cat. 50 mgm./kgm. acetazolamide was then given intravenously, and 3 hr. later samples were taken from the other ear. Electrolyte and drug concentrations were then determined.

At the time of removal of the samples, the plasma concentrations of acetazolamide ranged from 17 to 65 μgm./ml. The tissues of the inner ear were found to contain drug in the same range, averaging 30 μgm./gm. of tissue or 2×10^{-4} molar. From the values of enzyme units given in Table 1 and the corresponding molar concentrations of enzyme (E) which may be computed from other work in this laboratory[15], it is found that the enzyme concentration in these tissues ranged from about 1 to 6×10^{-5} molar. The K_I of acetazolamide is approximately 1×10^{-8} molar. From these values it may be calculated that the concentrations of acetazolamide found in the tissues are enough to inhibit more than 99·9 per cent of the enzyme originally present.

It was of interest in these experiments that, following the administration of acetazolamide, the volumes and pressures in the perilymph and endolymph appeared considerably reduced, a situation comparable to that reported for cerebrospinal fluid[4] and aqueous humour[1].

Carbon dioxide. The typical renal effect of acetazolamide was elicited, and the animals, at the time the samples were taken, had a definite metabolic acidosis. While untreated cats in the series had average total carbon dioxide values in plasma of 17 m*M*/l., after drug administration the values dropped to 11 m*M*/l. In five normal cats, values for the carbon dioxide of the endolymph ranged from 15 to 21 m*M*/l., and were not significantly different from those of the plasma and cerebrospinal fluid simultaneously sampled.

Table 3

Ref. 13. Cat		
Potassium	117·0 (12)*	

* In many of these samples the sodium and potassium concentrations suggested contaminations with perilymph. Three samples, however, showed the high values for potassium given in the table. The bracketed figure appended to each entry represents the total number of observations on which it is based.

Present study cat		
Controls	9, 20, 51, 55, 85, 123, 127	Av. = 67
15 hr. after acetazolamide	15, 21, 13, 2, 16*, 26†, 10†	Av. = 15

Potassium concentration in cochlear endolymph (m.equiv./l.) of cat.

* 3 hr. cat; † 6 hr. cat.

Chloride. Results for endolymph are preliminary in nature since only six samples were obtained. In two normal samples, the concentration of chloride in the endolymph was higher than that of the perilymph. Our results suggest that following acetazolamide, endolymph chloride decreased, while perilymph chloride may have increased. There does not appear to be any significant change in chloride concentration in either the plasma or the cerebrospinal fluid.

Potassium. Administration of acetazolamide appeared to eliminate all the high values of potassium concentration in the endolymph, and gave average potassium ion values which are strikingly less than the overall values of endolymph reported in our controls and for those of the workers previously cited (Table 3).

Perilymph potassium concentrations following the drug (Table 2) were in the same range as those from control animals. In the few cases studied, the values for cerebrospinal fluid were in the normal range.

These results suggest that carbonic anhydrase may play an important part in the mechanism of active secretion of potassium into the endolymph, and possibly in the overall formation of this fluid. In view of current theories regarding the etiology of Ménière's disease as a 'hydrops' of the inner ear, the obvious analogy to hydrocephalus and glaucoma suggests a rational basis for a controlled trial of acetazolamide for this syndrome.

This investigation was aided by a research grant (*B*-2242) from the U.S. Public Health Service.

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LABILITY OF N-*o*-CARBOXYPHENYLRIBOSYLAMINE AS A FACTOR IN THE STUDY OF TRYPTOPHAN BIOSYNTHESIS

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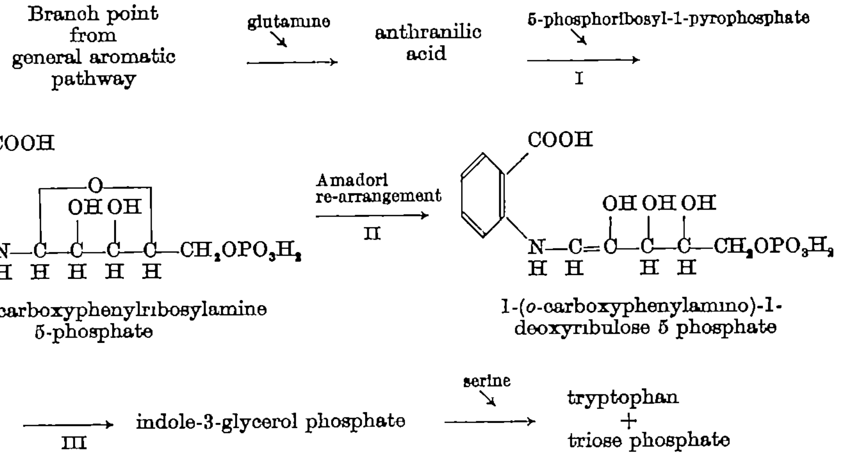
INVESTIGATIONS with both whole cells[1] and cell extracts[2] have established 1-(*o*-carboxyphenylamino)-1-deoxyribulose 5-phosphate as an intermediate in tryptophan biosynthesis. The likely sequence of reactions is:


Branch point from general aromatic pathway
glutamine
anthranilic acid
5-phosphoribosyl-1-pyrophosphate
I
COOH
O
OH OH
N—C—C—C—C—CH₂OPO₃H₂
H H H H H
N-o-carboxyphenylribosylamine 5-phosphate
Amadori re-arrangement
II
COOH
OH OH OH
N—C=C—C—C—CH₂OPO₃H₂
H H H H
1-(o-carboxyphenylamino)-1-deoxyribulose 5 phosphate
III
indole-3-glycerol phosphate
serine
tryptophan
+
triose phosphate

The relation of anthranilic acid to tryptophan biosynthesis has recently been discussed in some detail[3]. Mutants blocked in reaction II would normally be expected to excrete N-*o*-carboxyphenylribosylamine (the dephosphorylated form of the postulated intermediate), but preliminary data based on the Bratton and Marshall[4] colour reaction suggested that spontaneous hydrolysis would result in the accumulation of large quantities of anthranilic acid. In this event such mutants would appear to be blocked in reaction I.

Since the Bratton and Marshall method requires the use of acid conditions which could favour the formation of artefact anthranilic acid, the lability factor was re-examined by a new method not requiring the manipulation of *p*H.

In Fig. 1 the ultra-violet absorption spectrum of anthranilic acid is compared with that of N-*o*-carboxyphenylribosylamine under equivalent conditions in a typical biological medium. Since the differential at 247 mμ is present over the *p*H range 6·0–9·0, this was used to follow the reaction: N-*o*-carboxyphenylribosylamine $\xrightarrow{H^+}$ anthranilic acid without the necessity of adjusting the *p*H of experimental samples. The general method was as follows. A 0·005 *M* solution of N-*o*-carboxyphenylribosylamine (or other anthranilic acid glycosylamine) was prepared in 0·05 *M tris* buffer, *p*H 9·0. A 1-ml. portion of this solution was added to 49 ml. of experimental buffer (for experiments at 37° this was pre-heated) to give a final glycosylamine concentration of 0·0001 *M*. The zero-time sample was withdrawn immediately and the bulk of solution placed in a water-bath at the experimental temperature. Further samples were withdrawn as required. The absorbance of all samples was read at 247 mμ (ultra-violet spectrophotometer) without delay.

For the *p*H range 6·0–7·0 the experimental buffer was Davis minimal medium[5] adjusted when necessary with potassium dihydrogen phosphate; in the range *p*H 7·5–9·0 the buffer was 0·1 *M tris*.

At 100 per cent conversion to anthranilic acid there was excellent agreement with the known overall ultra-violet absorption of anthranilic acid (Fig. 1), while at intermediate stages the spectra were consistent with the appropriate mixture of anthranilic acid and glycosylamine derivative.

As shown in Table 1, the half-life of N-*o*-carboxyphenylribosylamine is dependent on *p*H. Media used in accumulation studies with tryptophan auxotrophs are rarely buffered at *p*H-levels greater than 8. Unless special precautions are taken, the buffer is broken and it is not uncommon for the *p*H to fall to between 5 and 6. Even without this drop the

Table 1. STABILITY OF N-*o*-CARBOXYPHENYL-D-RIBOSYLAMINE (0·0001 *M*) AS A FUNCTION OF *p*H AT 37°

*p*H	Half-life (hr.)
6·0	0·1
7·0	0·5
7·8	2·1
8·0	3·2
8·4	6·0
9·0	20·0 (approx.)

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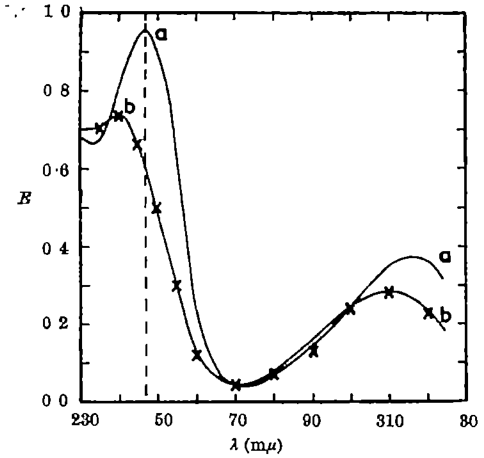

Fig. 1. Absorption spectra of: (*a*) N-*o*-carboxyphenyl-D-ribosylamine; (*b*) anthranilic acid, both 0·0001 *M* in Davis minimal medium, *p*H 7·0. The analytical method for anthranilic acid in the presence of its glycosylamine derivatives depends on the differential at 247 mμ. This differential is essentially the same between *p*H 6 and *p*H 9. ×, absorption of (*a*) at 100 per cent hydrolysis to anthranilic acid determined by E_{247} mμ

chemistry of the situation confirms that strains blocked in biosynthetic reaction II would form large quantities of anthranilic acid, a characteristic expected of strains blocked in reaction I. Strains blocked in reaction III are often referred to as anthranilic acid-accumulating strains. While mutants of this class do form anthranilic acid, appropriate examination of supernatants leaves no doubt that the Amadori derivative is the major product. In the case of mutants blocked in reactions I or II, anthranilic acid would appear as the major product of both types. Various experiments with whole cells[3] have not clarified the problem beyond a slight suggestion that mutants of type II might exist. It seems clear that whole cells are unsuitable for these investigations, principally because there is likely to be a permeability barrier to the phosphorylated glycosylamine. At the cell-free level the situation can be rationalized once the lability factor is taken into account. If both anthranilic acid and N-*o*-carboxyphenylribosylamine 5-phosphate are normal obligate intermediates, it is likely that tryptophan auxotrophs known to form anthranilic acid (in the strict sense discussed above) will eventually be shown to consist of two classes: (*a*) a class able to condense anthranilic acid with 5-phosphoribosyl-1-pyrophosphate; (*b*) a class deficient in this respect. It is also possible that the Amadori re-arrangement step is non-enzymatic.

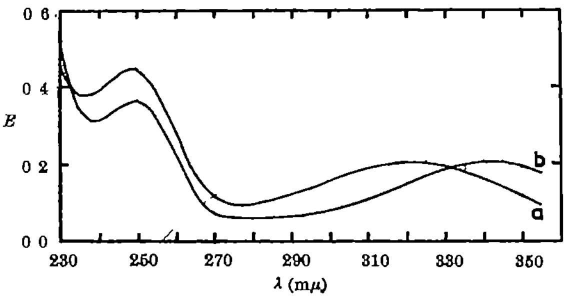

Fig. 2. Absorption spectra of crude 1-(*o*-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate. (*a*) 0·0001 *M* in buffer *p*H 7·0 (potassium phosphates 0·1 *M*); (*b*) *p*H adjusted to < 2 with conc. hydrochloric acid

With cell extracts the lability factor to be considered is that of N-*o*-carboxyphenylribosylamine 5-phosphate, not that of the dephosphorylated artefact. The phosphorylated version has yet to be synthesized and is therefore not available for study. Unsuccessful syntheses (Doy, C. H., and Doy, F. A., unpublished) based on successful methods for the nonphosphorylated glycosylamines of anthranilic acid[1] suggest that the phosphorylated form is more labile. A dividend from this work was the isolation of a crystalline compound with properties expected for the phosphorylated Amadori intermediate, 1-(*o*-carboxyphenylamino)-1-deoxyribulose 5-phosphate. Although Doy and Gibson[1] and Smith and Yanofsky[2] have described preparations of the non-phosphorylated and phosphorylated Amadori compound, a crystalline product has not been isolated previously. The present product has yet to be completely characterized, but its ultra-violet absorption at 10^{-4} *M* (Fig. 2) has the expected characteristics. On the basis of previous work, the extinction is of the order predicted. In contrast, Smith and Yanofsky[2] describe similar absorption, but at 10^{-3} *M*. The present measurement is based on weighings calculated from the formula $HOOC.C_6H_4.NH.CH_2.CO.(CHOH)_2.CH_2OPO_3Na_2$, while Smith and Yanofsky[2] base their spectrum on a concentration determined by enzymatic conversion to indoleglycerol phosphate. Since concentration based on weighing is more direct and impurity would be expected to shift maxima or depress extinction, the enzymatic data seems in some doubt.

The purely chemical observations suggest that N-*o*-carboxyphenylribosylamine 5-phosphate is (*a*) more easily hydrolysed to anthranilic acid than the non-phosphorylated form, and (*b*) easily converted to the Amadori compound. The first point would re-emphasize the lability factor in cell-free studies, and the second the possibility that the Amadori re-arrangement is non-enzymatic.

Since manipulations can be reduced to the minimum, fluorescence seems to be the most suitable means of looking for reaction I with cell-free systems. Methods which require the precipitation of protein with trichloracetic acid, or extraction procedures at acid *p*H, ignore the lability factor.

Smith and Yanofsky[2] have applied fluorometry to cell-free studies with anthranilic acid-accumulating tryptophan auxotrophs. Excluding those that were blocked immediately after the Amadori compound (Tryp-3*a* mutants), the remainder (Tryp-3*b* mutants) were classed as blocked immediately after anthranilic acid on the basis of failure to metabolize anthranilic acid. Since the reference describing the fluorometric method requires samples to be transferred to *p*H 6·0, it is possible that the lability factor has obscured the metabolism of anthranilic acid to its ribosylamine 5-phosphate. Similarly, experiments with radioactive substrates do not exclude the possibility of hydrolysis following enzymatic synthesis.

It is not clear whether 'mixing experiments' were done within the Tryp-3*b* class. If individual mutants within this class were point mutations lacking in one instance the enzyme necessary for reaction I and in another the enzyme necessary for reaction II, they would be expected to complement each other in the way described[2] for extract mixtures of Tryp-3*a* and Tryp-3*b* mutants. Such complementation would suggest a subdivision within class Tryp-3*b* mutants without the need to measure N-*o*-carboxyphenylribosylamine 5-phosphate directly.

If experiments can be designed which adequately account for glycosylamine lability, it should be possible to decide finally the question of whether some, all, or none of the anthranilic acid-accumulating strains are blocked in the Amadori re-arrangement step. It will then be easier to decide whether anthranilic acid has the status of a normal obligate intermediate.

This work was for the most part completed in the Bacteriology School, University of Melbourne, and supported by the Australian National Health and Medical Research Council. Work at Harvard was supported by a Fulbright Research Fellowship and a Commonwealth Scientific and Industrial Research Organization Overseas Post-Graduate award.

Note added in proof. Enzymatic results have now been obtained which are consistent with the division of anthranilic acid-accumulating mutants into a group blocked in reaction I and a group blocked in reaction II (Doy, C. H., Rivera, A., and Srinivasan, P. R., *Biochem. Biophys. Res. Comm.*, in the press).

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LOCATION OF THE NON-LINEARITY IN HORIZONTAL CELL RESPONSE TO RETINAL ILLUMINATION

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IN 1953, Svaetichin[1], using micropipette electrodes, found graded responses to light stimuli in isolated fish retinæ. Starting from a negative membrane potential of 10–50 mV., a graded hyperpolarizing signal, termed the 'luminosity response', could be obtained. The relationship between response and light intensity was found to be non-linear. As will be shown, it would appear that the receptor process is linear, and that the non-linearity is probably located in the region of a horizontal cell.

In recent work[2], we have definitely localized the site of origin of the luminosity response by the use of a marking technique[3] and subsequent histological examination. The response was found to originate in horizontal cells located in the bipolar cell layer of the retina. These cells are comparatively large, about 100μ across, and of quite low resistance (probably less than 100 kΩ (ref. 4)). On the basis of ultrastructural studies[5], these horizontal cells are considered to be glia, non-neuronal structures which are apparently much more permeable to sodium than to potassium[6]. Further, retinal tissue contains roughly equal amounts of sodium and potassium[7], neurons and glia are closely packed[5] (100-Å. spacing), and the 'extraneuronal' space is probably about equal to the neuronal space[6]. It is therefore likely that in glia cells the concentration of sodium must be considerably greater than that of potassium.

The non-linearity in horizontal cell response can be exhibited in two ways. Svaetichin *et al.*[8] showed that if the illumination per unit area is kept constant, but the illuminated area increased, the response increased with the number of cones thus illuminated, as shown in Fig. 1. It will be noticed that if the number of cones is not too small, the response increases as the logarithm of the number of cones. If the illuminated area remains constant, but the light intensity is increased, the response increases as the logarithm of the light intensity, provided the illumination is not too low. This is shown in Fig. 2. In this case a large number of cones were illuminated.

The fact that the curves in Figs. 1 and 2 have similar semi-logarithmic forms is explained by the following experimental result: The retina is illuminated by two light spots of the same diameter and of equal intensity, in one case overlapping, and in the other case just touching. The ratio of the response with two spots to that with one spot illuminated is found to be the same under both conditions. A typical result for Centropomidae is shown in Fig. 3, each spot illuminating about a hundred cones. In fact, if the two spots are non-overlapping and the effective intensity taken as the sum of the intensities of the

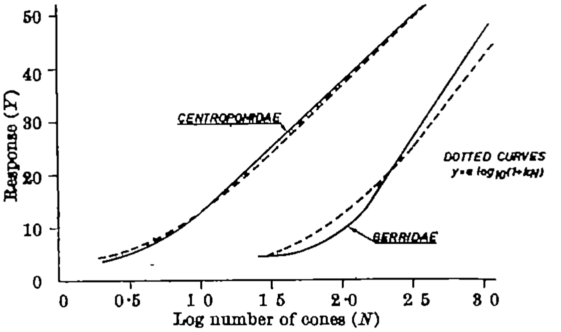

Fig. 1. Horizontal cell response as a function of the number of cones illuminated

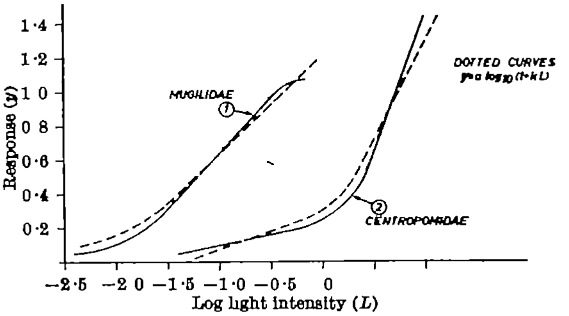

Fig. 2. Horizontal cell response as a function of the retinal illumination. Zero on the horizontal scale corresponds to different light intensity values for curves (1) and (2)

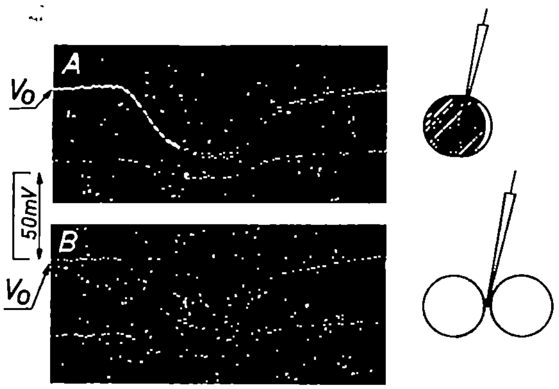

Fig. 3. Luminosity response in Centropomidae, overlapping (A) and adjacent (B) light spots of equal intensity. V_0 is resting potential of the horizontal cell. Duration of light stimulus is 350 msec. Top traces in (A) and (B) show response to single light spot; bottom traces show response with one spot permanently illuminated and the other switched on and off

two spots, the response is similar to the Centropomidae curve in Fig. 2. Hence, since two different groups of cones are involved when the light spots are separated, the non-linearity which affects the horizontal cell response cannot be in the receptors themselves, but must be where their signals converge. This is shown schematically in (A) of Fig. 4. Considering Fig. 1, it now follows that if the number of illuminated cones are increased from N_1 to N_2, with a fixed light flux per cone, the response is that which would be obtained by keeping the number of illuminated cones fixed at N_1 but increasing the illumination in the ratio N_2/N_1. Thus, the curves in Figs. 1 and 2 may be expected to have similar semi-logarithmic shapes, since Fig. 2 was obtained with a fixed light spot diameter.

The physiological system involved is more in accordance with (B) of Fig. 4, since the bipolars which receive signals from the receptors are in close proximity to the horizontal cell the electrical response of which is measured by means of a micro-electrode. It may therefore be expected that the non-linearity is subsequent to the summated actions of the bipolars on the horizontal cell. It is tempting to go further and assume that the horizontal cell potential is associated with a concentration difference of a particular ion i between the inside and the outside of the cell, the 'outside' of the cell being presumably the extremely narrow intercellular spaces of approximately 100 Å. width which are seen in high-resolution electronmicrographs[5]. The resting potential of the horizontal cell is then :

$$V_0 = A \log_{10} \frac{i_0}{i_i} \tag{1}$$

where i_i and i_0 are respectively the inside and the outside concentrations of the ion i, and A is a constant.

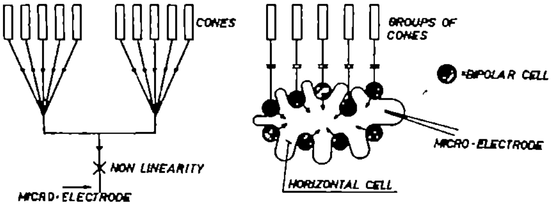

Fig. 4. Location of the non-linearity in horizontal cell response to retinal illumination

We assume that when the cones are illuminated, and equilibrium conditions have been established, a quantity of ions i proportional to the illumination L has passed from the bipolars into the horizontal cell. The horizontal cell potential is therefore increased to :

$$V_L = A \log_{10} \frac{i_0}{i_i + b.L} \tag{2}$$

where b is a constant. It is assumed that the ion concentration i_0 in the 100 Å. intercellular space remains unchanged. Hence, the response to light, as measured by the change in resting potential, is :

$$V_L - V_0 = -A \log_{10}(l + k.L) \tag{3}$$

where k is a constant equal to b/i_i. The dotted curves in Figs. 1 and 2 are of the form given in equation (3), and there is at least a fair fit with the experimental results. However, there is difficulty in accepting this simple interpretation of the source of the non-linearity. The horizontal cell is large, about 100μ diameter, and diffusion of sodium or potassium ions throughout the cell would take a time of the order of 700 msec., using the Hodgkin and Keynes[9] measurements of diffusion-rates in squid axoplasm. But only about 50 msec. elapses between application of illumination and attainment of 80 per cent of the maximum response in the horizontal cell, a time which also includes transmission from the receptors to the bipolar cells (a distance of about 60μ in the fish). Possibly the ionic influx from the bipolars spreads along the cell in some way which involves electric field gradients. It is perhaps significant that if the fish is dark adapted, the luminosity response still follows a similar curve to equation (3); however, the response now has very slow rise and decay phases about 1 sec. (ref. 10), so that this may indicate a diffusion process. In any event, it would certainly appear that the non-linearity in response is closely connected with the horizontal cell; there is, at least, formal correspondence between the response and a hypothetical mechanism which involves the passage of ions between bipolars and the horizontal cell, and the associated change in ionic concentration ratio between the interior and the exterior of the horizontal cell.

Finally, it is interesting that the psychological scale of lightness[11] can be fitted quite well by a relationship of the form of equation (3), while it is well known that in the retina of fishes, etc., there are ganglion cell units where the relationship between spike frequency and illumination is also of a similar form. It is reasonable to suppose that the change of bipolar cell potential in response to light can be represented by equation (3), and that the spike frequency is linearily related to this graded potential as has been shown, for example, by Fuortes[12] in *Limulus*.

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FOUR ADULT HÆMOGLOBIN TYPES IN ONE PERSON

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THE normal adult type of human hæmoglobin (A) is a single molecular species with the chemical constitution $\alpha_2{}^A\beta_2{}^A$ (ref. 1). Such a molecule contains two identical α polypeptide chains and two identical β chains. It has been shown that the genetically controlled abnormal human hæmoglobins previously examined contain an abnormal α or an abnormal β chain, carrying an amino-acid substitution[2,3]; for example, lysine for glutamic acid in the β chain of hæmoglobin C, $\alpha_2{}^A\beta_2{}^C$ (ref. 4). We wish to report the results of chemical studies on four adult hæmoglobin components[5] found in a single individual, D. K. P.; her hæmoglobins turned out to be normal in both peptide chains, abnormal in one or other chain and abnormal in both chains, respectively. Apart from their genetic interest, these findings allow us to make certain deductions about the mode of assembly of the hæmoglobin molecule.

The blood sample from this patient, D. K. P., became available to us through the kindness of Dr. L. M. Tocantins and Miss J. Atwater of Philadelphia. They have already described[5] the occurrence of four hæmoglobins in approximately equal amounts in this blood, together with their clinical findings. The hæmoglobins are electrophoretically distinct at *p*H 8·6; they have been called[5] hæmoglobins A, G, C and X, which is the order of migration, with A as the fastest component. Electrophoretic comparison with authentic specimens is the basis for naming the first three hæmoglobins; hæmoglobin X was unknown and the slowest hæmoglobin yet described. The hæmoglobin G was found by these authors[5] to behave identically with a specimen of G from Dr. Schneider. It may therefore be identical with the hæmoglobin G of Lehmann and Edington[6] and with the G_{Ib} (for G_{Ibadan}) recently described by Shooter *et al.*[7], and found by them to be abnormal in the α chain. On the other hand, D. K. P.'s hæmoglobin G is different from the G of Schwartz *et al.*[3,8] which carries a substitution of glycine for glutamic acid in the β peptide chain.

The original characterization of hæmoglobins A, G, C and X was done by paper electrophoresis[5]. We have been able to confirm the presence of the four components by starch-gel electrophoresis[9] at *p*H 8·6 in 0·02 *M* veronal buffer (see Fig. 1). The four proteins were also separated by electrophoresis on a starch block[10] in the same buffer. The relative amounts (per cent) recovered were: A, 35; G, 27; C, 23; X, 15.

Each hæmoglobin was digested with trypsin and fingerprinted[11]. Hæmoglobin A appeared to be completely normal (Fig. 2) and hæmoglobin C showed only the expected absence[4] of peptide 4 and its replacement by two new peptides, C-4*a* and C-4*b* (C-4*b* = hæmoglobin C tryptic peptide 4*b*). These peptides were isolated, hydrolysed and their amino-acid content analysed. The results confirmed the glutamic acid to lysine substitution previously reported for hæmoglobin C[4]. It appears, therefore, that D. K. P.'s hæmoglobin C is authentic hæmoglobin C.

Fingerprints[11] of D. K. P.'s hæmoglobin G were more difficult to interpret. The fingerprinting technique had to be modified to make it more sensitive. Isolated peptide bands from paper ionophoresis at *p*H 6·4 are compared side by side by a second ionophoresis on Whatman No. 3*MM* paper in a *p*H 4·7 buffer. It eventually appeared that in hæmoglobin G, two normal peptides—A-3 and A-9—had been replaced by three new peptides. No other changes have so far been detected.

The following relationship for these five peptides was established by means of quantitative amino-acid analyses of the isolated peptides with the Spinco model Moore and Stein automatic analyser, by end-group determinations with the dinitrophenol procedures[12], by stepwise degradation with the Edman–Sjøquist procedure[13] and by fingerprinting[11] of some of the peptides after their digestion with elastase[14].

The hæmoglobin A peptides A-3 and A-9 differ only in that A-9 has an additional N-terminal lysine residue; we may write A-9 = Lys(A-3). The Lys-(A-3) bond in such a peptide would be attacked by trypsin only slowly; hence yields of peptides A-9 and A-3 were found to be variable.

(1) In D. K. P.'s hæmoglobin G, an asparagine residue in peptide A-3 (and A-9) has been replaced by lysine; hence a new trypsin-sensitive bond has appeared which results in the appearance of an additional tryptic peptide. Therefore we can write:

$$\text{A-3} \rightarrow \text{G-3}a + \text{G-3}b$$
$$\text{A-9} \rightarrow \text{G-9}a + \text{G-3}b (= \text{G-9}b).$$

Of course, peptide G-9*a* = Lys-(G-3*a*), as illustrated in Fig. 3.

(2) It so happens that peptide G-3*b* contains methionine, which in the course of fingerprinting is

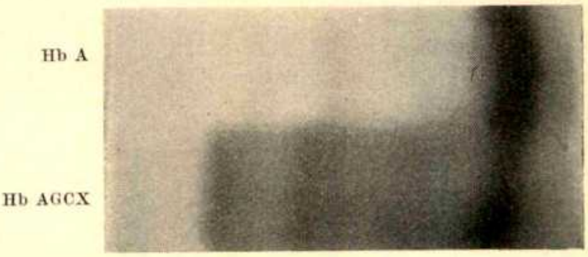

Fig. 1. Starch-gel electrophoresis of D. K. P.'s hæmoglobin at *p*H 8·6 in 0·05 *M* veronal buffer

to some extent oxidized to the sulphoxide. The resultant peptide 'G-3*b* ox' is definitely identifiable, since it is separated in chromatography. Peptides A-3 and A-9 (and for that matter A-25) show the same oxidation phenomenon.

Hæmoglobin X was also digested and fingerprinted[11]. It is clear from the results (Figs. 2 and 3) that hæmoglobin X contains the abnormal peptides characteristic of both hæmoglobin C and G. Peptide A-4 (β chain) is replaced by the two hæmoglobin C peptides C-4*a* and C-4*b*. Peptides A-3 and A-9 (α chain) are replaced by the three hæmoglobin G peptides G-3*a*, G-9*a* and G-3*b*. Hæmoglobin X is therefore a naturally occurring adult hæmoglobin which is abnormal in both its α and its β chains. Such doubly abnormal hæmoglobins have been prepared *in vitro* by Itano and Robinson[15] using dissociation and re-association of suitable mixtures of singly abnormal human hæmoglobins.

One can now write the constitution of D. K. P.'s four hæmoglobins as follows:

Hæmoglobin A = $\alpha_2^A\beta_2^A$

Hæmoglobin G = $\alpha_2^G\beta_2^A$

Hæmoglobin C = $\alpha_2^A\beta_2^C$

Hæmoglobin X = $\alpha_2^G\beta_2^C$

We would deduce from these findings that D. K. P.'s genotype is $\alpha^A/\alpha^G\ \beta^C/\beta^A$ (see also in refs. 16 and 17). It has also been suggested before by Atwater *et al.*[5] that D. K. P. is doubly heterozygous for the G and C abnormalities. Apparently, each gene causes the manufacture of the peptide chain characteristic of it; thus, the α^A gene produces α^A chains, the α^G gene produces α^G chains with lysine in place of asparagine in peptide A-3, and so on. We must next assume that each chain dimerizes as soon as it is formed; $2\alpha^A \rightarrow \alpha_2^A$ and $2\alpha^G \rightarrow \alpha_2^G$. This must be so, since no real hybrids, such as $\alpha^A\alpha^G\beta_2^A$, etc., are produced, although all these chains are present inside the same cells. Finally, the four types of subunit seem to assemble to complete molecules in a random fashion, leading to the four hæmoglobins:

$$\alpha_2^A + \alpha_2^G + \beta_2^A + \beta_2^C \rightarrow$$
$$\alpha_2^A\beta_2^A + \alpha_2^G\beta_2^A +$$
$$\alpha_2^A\beta_2^C + A_2^G\beta_2^C.$$

It is important to realize the random nature of this final step in the assembly of the molecule, in contrast to the previous dimerization step, which is specific. The fact that the four hæmoglobins occur in roughly equal amounts, with A predominating, implies that at least in this patient the rate of manufacture of the different chains is only slightly different. As in the other abnormal human hæmoglobins, the phenotype corresponding to each gene is expressed and the product of all of them is to be found within the same cell.

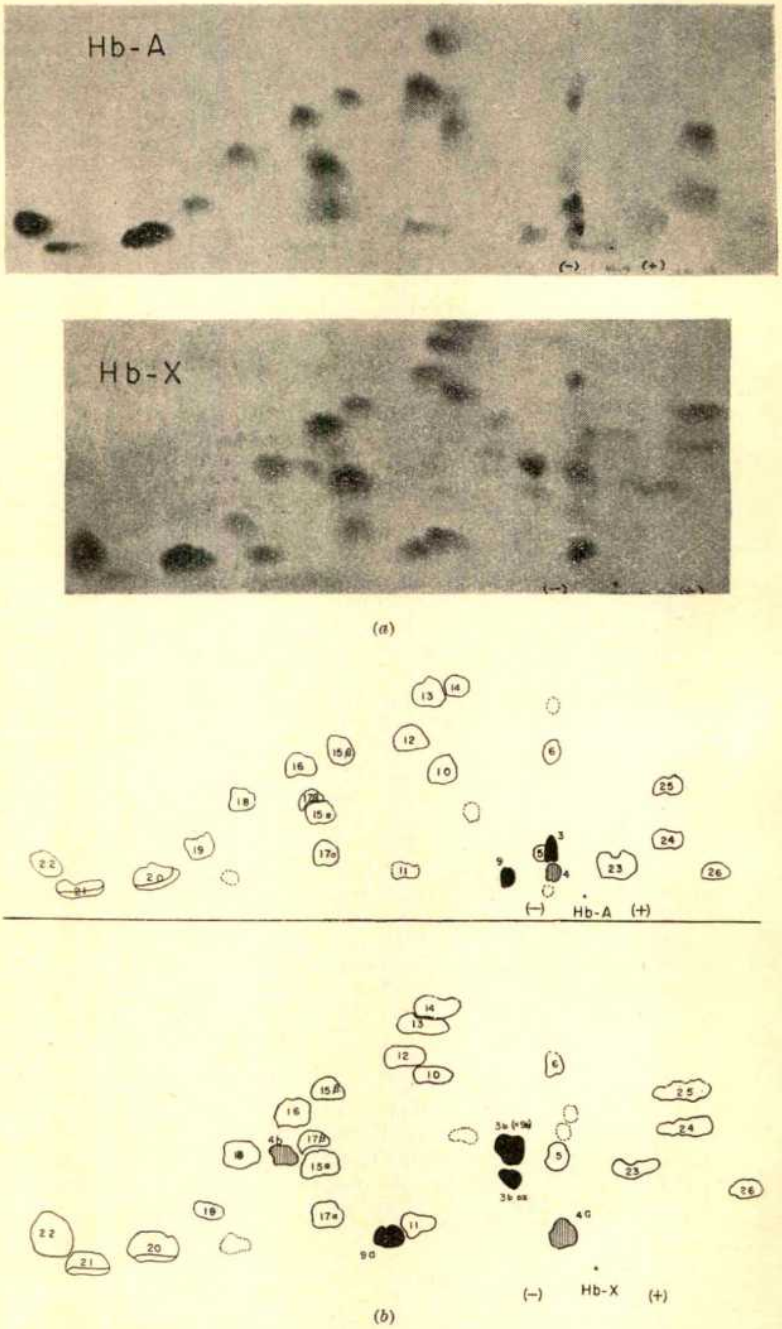

Fig. 2. Photographs (*a*) and tracings (*b*) of fingerprints (ref. 11) of hæmoglobins A and X. Electrophoresis was in the usual buffer but on a cooled brass plate at about 30 V./cm. for 2½ hr. Ascending chromatography is in pyridine : *iso*amyl alcohol : water—35 : 35 : 30 by volume (ref. 18). The methionine residue in G-3*b* is often partially oxidized to the corresponding sulphoxide peptide with a different chromatographic mobility. Such an oxidized peptide is referred to as 'G-3*b* ox'. Peptides A-4, C-4*a* and C-4*b* are marked by a vertical shading. Peptides A-3, G-3*b*, G-3*b* ox and G-9*a* are marked in black

Rather similar chemical results are being obtained by Dr. Park Gerald, Boston, working with an apparently unrelated patient who possesses four hæmoglobins called at present A, D, C and X. However, the four components are not nearly in equal amounts, hæmoglobins C and X greatly predominating. It will be interesting to see whether these hæmoglobins D and X are identical chemically with D. K. P.'s hæmoglobins G and X and why the proportions are so different. Very recently, Raper *et al.*[20] reported briefly on the occurrence of A, G, C, X in one patient, with X abnormal in both chains. The relationship between their hæmoglobins and ours remains to be determined.

Full details of our experiments have been submitted for publication.

We wish to thank Miss Jean Atwater and Dr. L. M. Tocantins for their help and interest. We would also like to acknowledge the help of Miss Marianne Schick, who performed the quantitative amino-acid analysis. This work has been supported by a grant from the Institute for Arthritis and Metabolic Diseases, U.S. Public Health Service.

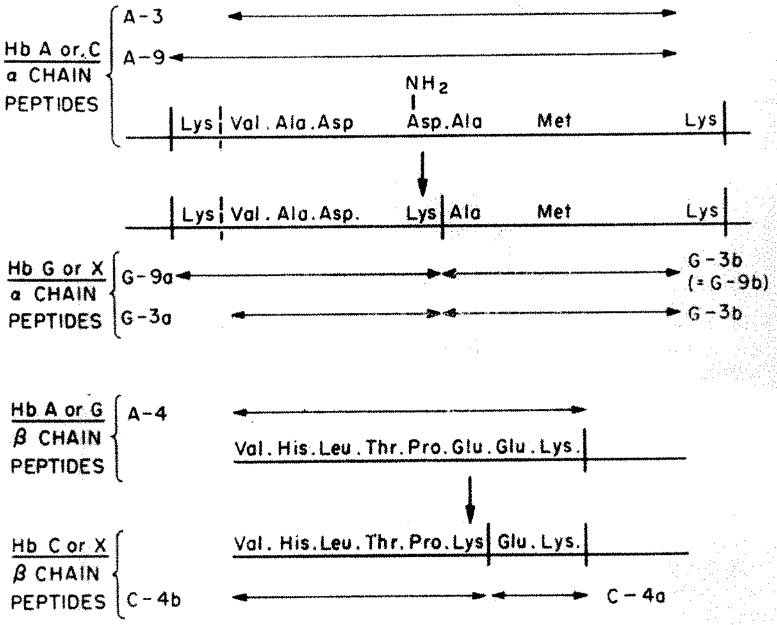

Fig. 3. Diagrammatic representation of the peptides obtained by the action of trypsin on the α- or β-chains of hæmoglobins A, G, C, X. See text for the explanation of the relationship between peptide A-3 and A-9 and between G-3*a* and G-9*a*. In the lower part of the figure it can be seen that peptides A-4 and C-4*b* are at the N-terminus of the β-chains (ref. 19) of hæmoglobins A and C. Peptide A-9 contains 29 amino-acid residues, peptide G-9*a* has 8

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GENETIC CONTROL OF FŒTAL AND ADULT HUMAN HÆMOGLOBIN

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ADULT human hæmoglobin (A) is known to consist of two types of polypeptide chain of roughly equal lengths[1]. The gross formula of this hæmoglobin may therefore be written as $\alpha_2\beta_2$, or more specifically as $\alpha_2{}^A\beta_2{}^A$, to indicate that the chains have the amino-acid sequences characteristic of hæmoglobin A. The synthesis of α and β chains is supposedly controlled by separate α and β genes[2]. Fœtal human hæmoglobin (F), on the other hand, has the constitution $\alpha_2{}^A\gamma_2{}^F$, indicating that this hæmoglobin differs in the structure of only two of its four peptide chains[3]. Since Hunt[4] has shown that the α chains of fœtal and adult hæmoglobin are similar, and probably identical, it became of interest to find out whether their production is controlled by the same α chain gene operating in the fœtus and in the adult. The investigations described in this article support the idea of control by the same α chain gene.

Hæmoglobin I was first described by Rucknagel *et al.*[5] as an electrophoretically fast abnormal hæmoglobin. An unrelated case of hæmoglobin I, abnormal in the α chain[6], has more recently been found in a coloured woman, J. W., by Schwartz *et al.*[7]. J. W. is unusual in possessing 70 per cent hæmoglobin I and 30 per cent hæmoglobin A, whereas usually the

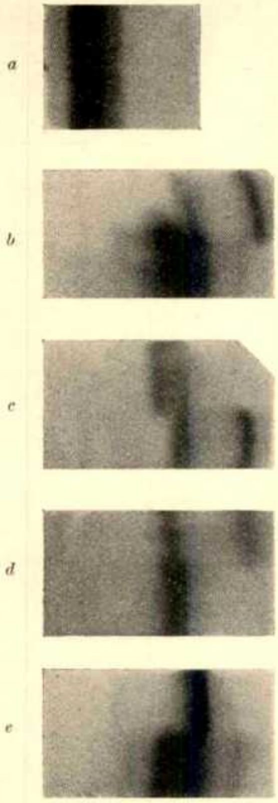

Fig. 1. Starch-gel electrophoresis (ref. 10) experiments in 0·02 *M* veronal buffer, *p*H 8·6. *a*, P. W., cord blood (upper sample) and blood with authentic hæmoglobin Bart's (lower sample); *b*, P. W., 1 day sample (lower) and hæmoglobin A + I from his mother, J. W. Overloaded to show the presence of Bart's in P. W. Note unexplained slow component in P. W., which is not hæmoglobin A_2; *c*, P. W., 2 months sample (upper) and hæmoglobin A + I. Hæmoglobin Bart's has largely disappeared from P. W.; *d*, P. W., 6 months (lower), showing absence of Bart's and fœtal hæmoglobin and hæmoglobins A + I; *e*, P. W., 1 day sample (lower) and 6 months sample

abnormal hæmoglobin comprises less than 50 per cent of the total. Atwater *et al.*[8] suggested that the explanation for this fact lies in J. W. being heterozygous for hæmoglobin I and for thalassæmia. On the basis of a recently developed hypothesis for the occurrence of the thalassæmia[9], we would picture J. W.'s *genotype* as:

$$\frac{\alpha^{I}\ \ \beta^{A}}{\alpha^{Th}\ \beta^{A}}$$

The gene α^{Th} would cause the production of an α chain carrying a type of amino-acid substitution not detectable by electrophoresis, but which nevertheless drastically reduces the rate of production of such chains. Hence would follow the relatively low amount of hæmoglobin 'A'—really $\alpha_2^{Th}\beta_2^{A}$—in the blood of J. W.

Through the courtesy of Dr. L. Tocantins and Miss J. Atwater, we have been able to examine the blood of J. W.'s recently born son, P. W. Examination of the cord blood, and of a specimen collected at 24 hr., by starch-gel electrophoresis[10] at *p*H 8·6 in 0·02 *M* veronal buffer showed the presence of a fast-moving hæmoglobin component, judged to be 10 per cent of the total, in addition to hæmoglobins A and F (Fig. 1). By this method of electrophoresis the fast component had the same mobility as hæmoglobin Bart's[11], a variant of fœtal hæmoglobin, but was considerably slower than J. W.'s hæmoglobin I. The same result was obtained by electrophoresis on a starch block[12] at *p*H 8·6 in 0·05 *M* veronal buffer. The fast component was isolated and dialysed against 0·04 *M* phosphate buffer *p*H 8·0. Its absorption spectrum was normal with peaks at 578, 543, 412 and about 355 mμ, but in the ultra-violet region there was a pronounced tryptophan peak at 291 mμ which is characteristic[13] of fœtal hæmoglobin (Fig. 2). Hæmoglobin Bart's is a fœtal hæmoglobin known[14] to have the constitution γ_4^F and to occur in the presence of thalassæmia[11].

The identity of baby P. W.'s fast hæmoglobin component with hæmoglobin Bart's was further demonstrated by comparing the behaviour of P. W.'s blood with an authentic specimen of hæmoglobin Bart's during ion-exchange chromatography on the carboxyl exchange *CG*50 type II according to Huisman and Prins[15] (Fig. 3). After development of the chromatogram for 30 min., hæmoglobins A and F were clearly separated. Ahead of them moved a well-defined hæmoglobin band at the same speed in the case of P. W.'s blood and in a specimen of hæmoglobin Bart's. Under these conditions, the hæmoglobin I moves between hæmoglobins A and F.

Baby P. W.'s fast hæmoglobin component, isolated by starch-block electrophoresis, was digested with trypsin and fingerprinted[16] alongside hæmoglobins Bart's and F. The pattern of tryptic peptides obtained from hæmoglobin Bart's and P. W.'s fast component showed the virtual absence of peptides 20 (α chain) 18, 9, 13 and 16 (ref. 15). These are characteristic of the α chain of human hæmoglobin. Hence, the fingerprints also suggest that this fast hæmoglobin has the constitution γ_4^F.

Recently, it was found that a precipitate had appeared in P. W.'s 24-hr. sample which had been stored frozen for a few months. At the same time, the fast hæmoglobin component had disappeared from the supernatant, which contained only hæmoglobins A and F. Hæmoglobin Bart's as well as hæmoglobin H (ref. 17)—β_4^A—are known to precipitate very easily. The precipitate from P. W.'s cord blood was fingerprinted by an improved method

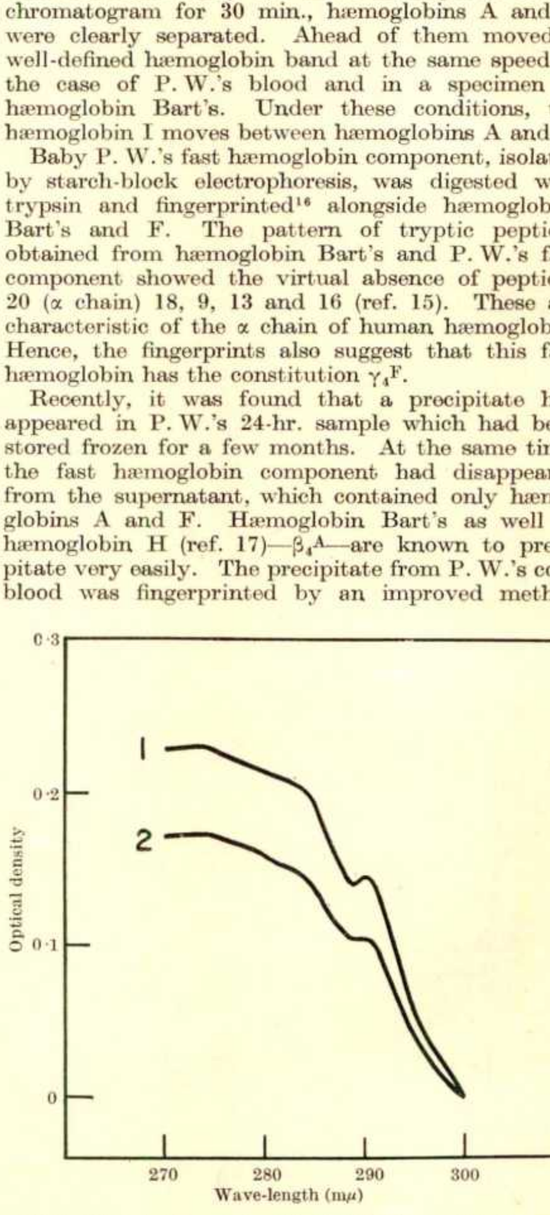

Fig. 2. Ultra-violet spectrum of P. W.'s fast hæmoglobin (1) and of hæmoglobin Bart's (2) isolated by starch-block electrophoresis (ref. 12)

(Fig. 4) and was seen to be largely composed of γ^F chains, as expected for hæmoglobin Bart's.

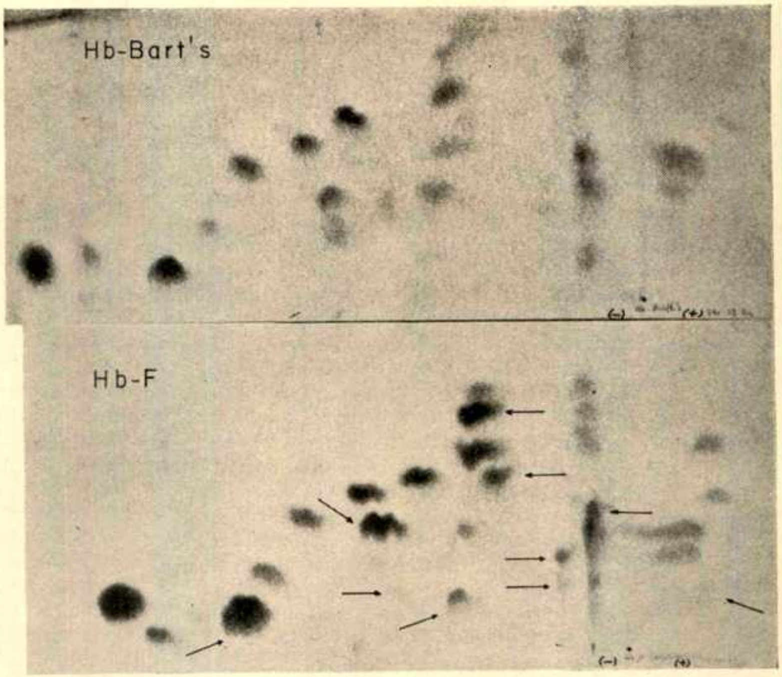

Fig. 4. Photograph of fingerprints (ref. 16) of precipitated hæmoglobin Bart's from baby P. W. and of hæmoglobin F. Electrophoresis was carried out at *p*H 6·4 on a water-cooled brass plate 24 in. long at 30 V./cm. for 2¼ hr. Chromatography was in pyridine : *iso*amyl alcohol : water—35 : 35 : 30 by volume (Wittmann, H. G., personal communication). α-Chain peptides of hæmoglobin F, as indicated by arrows, are largely absent in P. W.'s hæmoglobin Bart's

The fast component in P. W.'s blood had virtually disappeared at six months, as expected for a fœtal hæmoglobin[13].

These results indicated that baby P. W. possesses hæmoglobin Bart's —γ_4^F—but not the hæmoglobin I of his mother. As expected, baby P. W.'s hæmoglobin Bart's had disappeared by 6 months ; but it was not replaced by the corresponding adult hæmoglobin H, which has the constitution β_4^A. Hæmoglobin H, like hæmoglobin Bart's, is usually associated with thalassæmia. Although one might expect *a priori* that the conditions which lead to hæmoglobin Bart's in the baby would also cause the appearance of hæmoglobin H in the adult, this is not necessarily so. Cases have been reported both in favour[18] and against[19] this expectation. The reasons for this variability are not clear.

Baby P. W. showed at 9 months a hypochromic microcytic blood picture[8]. It is reasonable to assume that baby P. W. inherited the α chain thalassæmia gene which is active in his mother, J. W., and which causes a decrease in the production of α peptide chains[9]. In turn, this situation would be expected to lead to an excess of γ chains, or rather γ_2^F dimers, which would then aggregate to form hæmoglobin Bart's. Baby P. W.'s genotype would be :

$$\frac{\alpha^{Th}}{\alpha^{A}}\ \frac{\beta^{A}}{\beta^{A}}$$

The idea that the same α chain is concerned in the production of the adult and the fœtal hæmoglobin is an important one, since it allows us to formulate more clearly the probable mechanism involved in the switch from fœtal to adult protein. It is likely[20] though not undisputed that at the time of birth both hæmoglobins are present within the same cell. In that event, and in view of the findings reported above, we picture the change-over mechanism as involving the switching down of the activity of the γ chain gene and the switching on of the β chain gene, while the α gene remains active all the time.

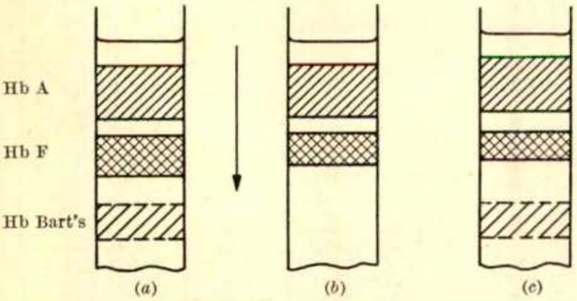

Fig. 3. Column chromatography (ref. 15) of P. W.'s hæmoglobin (*a*), of normal cord blood (*b*) and of blood with authentic hæmoglobin Bart's (*c*)

We wish to thank Dr. L. M. Tocantins and Miss Jean Atwater, Philadelphia, for their encouragement and for their generous help which made this investigation possible. Dr. H. Lehmann, London, generously supplied a sample of hæmoglobin Bart's. We would also like to acknowledge the many stimulating discussions with Dr. Park Gerald, Boston. This work has been supported by grants from the National Science Foundation and from the Institute for Arthritis and Metabolic Diseases, U.S. Public Health Service.

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EVIDENCE OF ACTIVE ION TRANSPORT ACROSS LARGE CANINE BLOOD VESSEL WALLS

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PREVIOUS work by members of this group has shown that disorders of the flow of blood through both large and small blood vessels appear related to induced or spontaneous electrical currents or their related electrical potential differences in either the vessel wall or surrounding tissues[1-3]. Blood cells, including platelets and blood proteins, are cataphoretic in an electrophoretic cell at the *p*H of blood[4-6]. It appears possible that abnormal electric currents produce local positively charged electrophoretic areas or other disturbance of the ionic atmosphere at the blood–intimal interface of damaged vessels; that these positive areas attract negatively charged blood cells to the blood vessel, resulting in intravascular thrombosis[7,8].

A correlated hypothesis suggests that the blood vessel wall under normal conditions expends energy moving ions to maintain the intimal–blood interface potential close to zero in a stable equilibrium. The measured transmural potential difference *in vivo* and *in vitro* appears to be very close to zero[7,8]. Experiments were designed to determine whether evidence for active ion fluxes across the blood vessel wall could be demonstrated.

These investigations were carried out using ion tracer techniques for the study of ion fluxes across membranes evolved by Dr. Hans Ussing[9] and others. Flux cells were modified after Hogben's design[10]. Fresh canine thoracic aorta or vena cava was opened along the long axis and divided into four equal samples of membrane. The segments were placed between four diffusion cell pairs so that each segment of wall formed a membrane 1 cm.² bathed on each side with oxygenated Krebs serum saline substitute[11] at 37° C. Tracer amounts of radioactive isotopes, 1 μc. of sodium-22, or sodium-24, and 1 μc. of chloride-36, were added to the intimal side in two of the cells and to the adventitial side in the remaining two cells to measure the rate of sodium, or chloride flux across the membranized blood vessel wall. One-ml. aliquots of the hourly removed recipient solutions were pipetted, dried and counted in a Geiger–Müller gas-flow counter. Flux was calculated in μmol./cm.²/hr. In selected experiments simultaneous trans-membrane potential differences were determined and compared with the measured ion fluxes across the blood vessel wall *in vitro*.

Ion flux determinations have been made on: (1) fresh, intact aortic wall; (2) aortic wall removed and stored for one or two days in Krebs serum saline substitute at 4° C.; (3) partitioned aortic wall, (*a*) the intima and subintimal layers in one cell compared with the (*b*) media and adventitial layers of the same segment of wall in a separate matching cell; (4) fresh vena cava wall. Only four of many groups studied will be dealt with in this communication.

Attempts were first directed at describing certain physical chemical characteristics of the blood vessel wall. Several groups of experiments resulted. These are: (1) Determination of the cumulative sodium flux across blood vessel wall for periods up to 6 hr. (2) The measurement of net hourly fluxes of sodium and chloride ions across aortic and vena caval wall to measure net ion transport (Fig. 1). (3) Determination of the oxygen dependency of the ion flux mechanisms in various experimental situations. These experiments fall into two groups. In the first group, matching pairs of aortic segments were exposed to ærobic and anærobic conditions (Table 1). In the second group of experiments segments of membranized aortic wall were exposed to sequential 2-hr. ærobic and anærobic periods (Fig. 2). (4) The determination of the temperature dependency of ion flux-rates.

Aorta. Dominant ion fluxes for both sodium and chloride occur from intima to adventitia across fresh, oxygenated, intact aortic wall membrane *in vitro* at a measured transmural potential difference very close to zero (approximate range, 0·3–2 mV.).

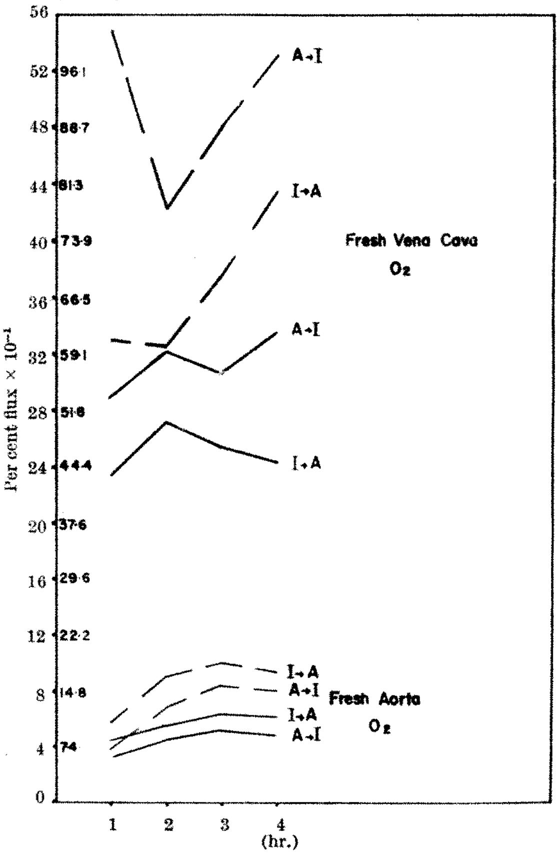

Fig. 1. Difference in rate of ion flux for membranized aortic and vena caval wall. Vena caval flux-rate is 4–5 times aortic flux-rate. *A*→*I* indicates flux from adventitia to intima; *I*→*A*, the reverse; O_2 indicates ærobic conditions. Chloride flux appears dominant for both vena cava and aorta. Vena cava dominant flux *A*→*I*; aortic dominant flux *I*→*A*
– – –, chloride-36; ——, sodium-24

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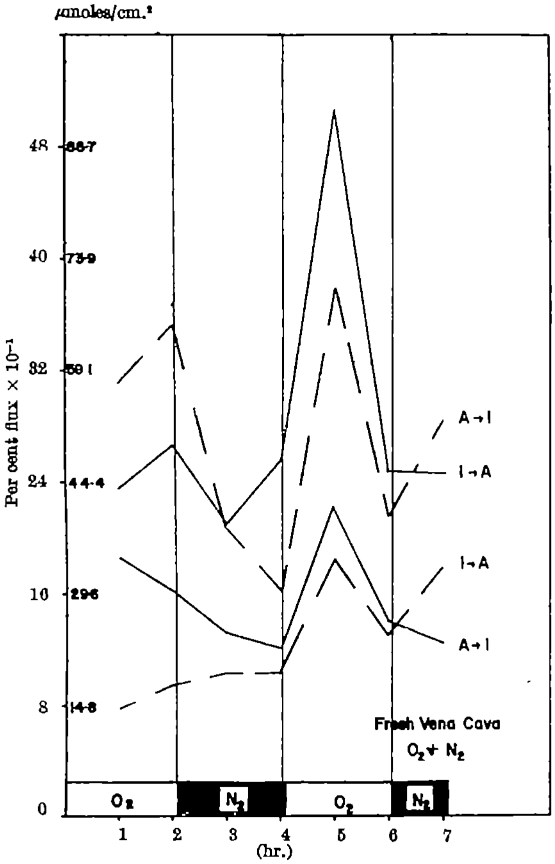

Fig. 2. The effect of consecutive ærobic and anærobic periods on sodium and chloride flux across vena caval wall. Under anærobic conditions sodium flux, $A \rightarrow I$, becomes dominant to chloride flux $I \rightarrow A$ in this experiment. The evidence indicates that sodium and chloride flux across vena caval wall is partially oxygen dependent, as shown for aortic wall in Table 1
– – –, chloride-36 ; ——, sodium-24

Vena cava. Sodium and chloride flux-levels for vena cava are five to six times those for the aorta (Fig. 1). This fact can be explained only in part by the greater thickness of the aortic wall. Both sodium and chloride show a mean net parallel flux. Note, however, that net flux across the aorta occurs in the opposite direction to that across the vena cava.

Ærobic versus anærobic ion transport (*Fig.* 2). In matched experiments in which two samples of membrane were exposed to oxygen and two samples of membrane to nitrogen, net ærobic sodium flux was usually opposite in direction to net anærobic sodium flux. The absolute magnitude of net fluxes was always different in the two groups, being greater under ærobic conditions (Table 1). Sodium and chloride ion fluxes across both aorta and vena cava show at least two types of marked change during sequential ærobic and anærobic periods. An absolute change in flux-rate occurs on switching from ærobic to anærobic conditions. Sodium frequently replaces chloride as the dominant ion transported across vena caval wall on establishment of anærobic conditions (Fig. 2).

Temperature effects. The information from experiments in which cell temperature was raised in steps indicates that the ion flux increases approximately 8–15 per cent per deg. C. rise from 8° to 56° C. The rate of flux increase per deg. C. closely parallels the changing rate of metabolic activity one might expect if sodium and chloride flux occurred across the blood vessel wall as the result of a metabolic process.

An Arrenhius plot of the rates of sodium and chloride ion flux indicates that the absolute rate of flux per deg. C. change varies critically at approximately 25° C. and again at 40° C. (Fig. 3).

Under the conditions of the experiment described, the greater part of ion movement across the blood vessel wall appears to be passive. Proof that all the ion movement is not passive is indicated by evidence presented in this article.

(1) Sodium and chloride fluxes across the membranized blood vessel wall are not identical in each direction.

(2) Net sodium and chloride ion movement across the aortic wall appears to be oxygen-dependent ; net flux frequently reverses onestablishment of anærobic conditions. The flux-rate change per deg. C. change in the temperature experiments and correlated Arrhenius plots also indicates active transport. By definition, both chloride and sodium flux in these

Table 1. SODIUM-22 FLUX ACROSS AORTIC WALL
Matched pairs of ærobic and anærobic determinations

Exp.	Gas	Direction of dominant flux		Non-heparinized Mean hourly flux μM/cm.²/hr.		*Net* flux direction	N_2–O_2	Comments
		$A \rightarrow I$	$I \rightarrow A$	$A \rightarrow I$	$I \rightarrow A$	$(A \rightarrow I)-(I \rightarrow A)$		–
64	N_2	X		14 78	10 2	+ 4 58	+ 4·27	Fresh
65	O_2		X	12·65	12·34	+ 0 31		
66	N_2	X		12·52	11 22	+ 1 30	+ 4·75	Aorta stored 1 day at 4° C.
67	O_2		X	11·46	14·81	– 3·45		
68	N_2	X		10·7	8·1	+ 2 60	+ 3·02	Fresh
69	O_2		X	11·23	11·65	– 0 42		
70	N_2		X	13·37	14·72	– 1·45	+ 1·20	Aorta stored 1 day at 4° C.
71	O_2		X	8·85	11·5	– 2 65		
78	N_2	X		14·7	11·6	+ 3 1	+ 4·70	Fresh
79	O_2		X	13·1	14 7	– 1 6		
					New series			
345	N_2	X		10 46	8·06	+ 2·40	+ 0 51	Fresh
344	O_2	X		8·44	6·55	+ 1·89		
347	N_2	X		12·42	6·83	+ 5·59	+ 6·71	Fresh
346	O_2		X	7·77	8·89	– 1·12		
349	N_2	X		16 89	11·90	+ 5 99	+ 8·45	Fresh
348	O_2		X	9 22	11 68	– 2·46		
							* t = 4·494 P = 0 001	

* t = Results of 't' test for statistical validity of results. P = Possibility that result is due to chance, in per cent.
Data from eight matched pairs of ærobic and anærobic experiments measuring sodium flux across the aortic wall. Both direction and rate of sodium flux vary under the contrasted conditions ($P < 0·001$).

Table 2. CALCULATED AND MEASURED POTENTIAL DIFFERENCES AS DETERMINED BY THE STANDARD DONNAN EQUILIBRIUM EQUATION

	Na-22, Na-24 experiments				Cl-36 experiments			
	f_i/f_0 mean	Calculated *PD*	Measured *PD*	No. of flux exps.	f_i/f_0 mean	Calculated *PD*	Measured *PD*	No. of flux exps.
Aorta	0·9193	+ 4·25 mV.	≈ 1·0 mV.	29	0·9726	+ 1·31 mV.	≈ 1·0 mV.	13
Vena cava	1·5634	– 10·11 mV.	≈ 1·0 mV.	13	1·5836	– 9·88 mV.	≈ 1·0 mV.	14

f_i = inward flux, adventitia to intima.
f_0 = outward flux, intima to adventitia.

experiments appear to be active transport, since the blood vessel wall or some of its layers bathed with identical solutions on each side move ions differentially at a measured potential difference close to zero. The results for the experiment for sodium and chloride flux exceed by 5–10 times the fluxes necessary to satisfy Donnan equilibrium equations at 37° C. (Table 2). For any univalent ion:

$$\frac{f_i}{f_0} = \frac{C_i}{C_0 Z}$$

$$C_i = C_0$$

$$\frac{f_0}{f_i} = Z$$

$$Z = \exp(Ef/RT)$$

$$Z_{37^\circ\text{C.}} = 10^{PD/0\cdot61}$$

$$PD = 61 \log Z_{37^\circ\text{C.}}$$

where *E* is potential difference (*PD*) in mV., *f* is Faraday's constant, *R* is gas constant, *T* is absolute temperature, if ion movement across the membrane is passive.

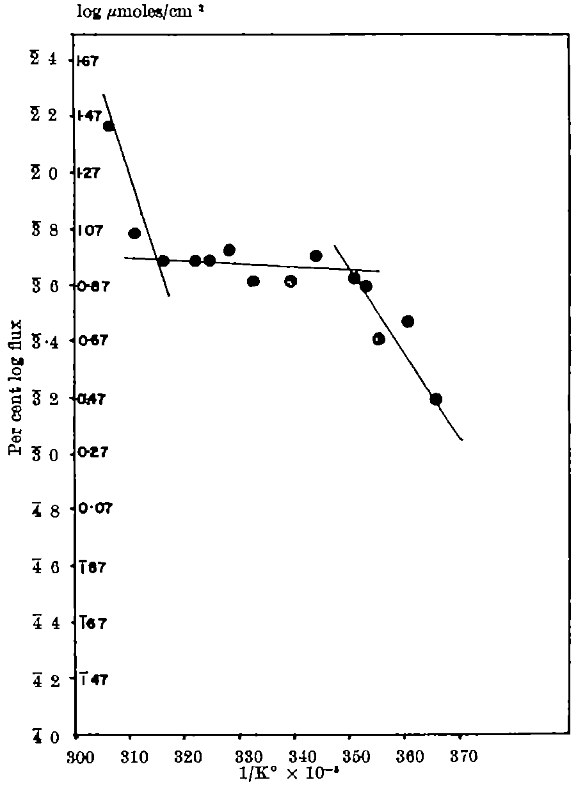

Fig. 3. An Arrhenius plot of sodium-24 flux-rate $I \rightarrow A$ across vena caval wall during which cell temperature was raised from 8° to 65° C. Log per cent flux is plotted to the left of abscissa, log μm./cm.²/hr to the right. The reciprocal of the absolute temperature is plotted on the ordinate

The results indicate that absolute change in flux-rate occurs as certain temperature-levels are reached

Net transport of one ion may occur at a very small transmural potential difference (1–2 mV.) under the conditions of this experiment only if equal amounts of another ion of equal and opposite sign accompany the measured ion across the experimental membrane, or an ion or ions of like or equal charge move in opposite direction across the membrane. Which of the two already-mentioned possibilities pertain to the situation described has still to be ascertained, for several other ions available to the vessel wall membrane could easily be transported during these experiments.

Do those experiments, performed *in vitro*, mirror phenomena which occur in living blood vessels *in vivo*? Evidence is available which indicates that brain capillaries do not fulfil Donnan equilibrium requirements and show evidence of active transport *in vivo*[12]. Whether the experiments described in this article relate to similar phenomena across smaller blood vessels and capillary walls has still to be determined.

Much has been conjectured concerning the fact that aortic and vena caval wall transport in opposite directions. Experiments and theory attempting to explain the difference will be offered in later publications.

Close to the blood intimal interface the net charge due to small net positive or negative ion transport across vessel walls may have a considerable though short-lived effect on interface potential differences. It would appear that tissue injury currents could derange this type of ion transport mechanism if of sufficient magnitude, and might result in reversal of net ion transport as is shown frequently in anaerobic experiments in this work. It is conceivable that vascular occlusion due to spontaneous tissue injury or due to applied small pseudo-injury currents might occur as a result of derangements in the flux mechanisms described here.

We wish to acknowledge the aid given by Dr. Adrian Hogben and Dr. Kenneth C. Cole in the course of these experiments.

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LETTERS TO THE EDITORS

GEOPHYSICS

Upper Limit for Interstellar Millicycle Gravitational Radiation

THE effect of gravitational radiation on an elastic body has been previously investigated[1]. The component varying with time of the Riemann tensor (R_{iojo}) associated with the gravitational field acts as a driving term in the wave equation for the strain or displacement. It was proposed[1] that the normal modes of the Earth or a laboratory mass be used as a detector of gravitational radiation. Experiments to measure the interstellar gravitational radiation at kilocycle frequencies are at present in progress at the University of Maryland.

From theoretical arguments we expect the interstellar gravitational radiation noise spectrum to show a peak at very low frequencies. It would therefore be advantageous to use a detector with as low a frequency response as possible. The largest mass available for an experiment is the Earth itself. The various theoretical modes for the vibration of the Earth have been known since 1882. However, it has not been until recently[2] that instruments with sufficiently long period have been available to measure the frequency and amplitude of these modes.

The data used were obtained from the N.32° W. Benioff strain seismograph at Isabella, California. The amplitude of the strain in the Earth's crust has been analysed from these data at the Seismological Laboratory of the California Institute of Technology, with the aid of the computational facilities at the Sandia Corporation[2]. Measurements taken after the recent Chilean earthquake were sufficient to identify the various vibrational modes and determine their breadth and frequency.

The data taken from the Isabella strain gauge during a quiet period do not show peaks at the frequencies of the various vibrational modes, hence the strains observed by the gauge are extraneous strain noise above the level of the actual strains in the modes during a quiet period. The data are therefore only useful for setting an approximate upper limit for the effects of gravitational radiation. Because of this, an exact analysis of the effect of the Riemann tensor on the various vibrational modes, although possible, would be of little value. For the purpose of calculating an upper limit, we shall use the equations obtained by Weber for the magnitude of the strains induced by the Riemann tensor in a longitudinally vibrating rod[1].

The strain in the elastic body at resonance is related to the Riemann tensor by:

$$\varepsilon(\omega) \approx \frac{2c^2 Q}{\omega^2 \pi} R_{iojo}(\omega) \qquad (1)$$

Here $\varepsilon(\omega)$ and $R_{iojo}(\omega)$ are the Fourier transforms of the strain and Riemann tensor, respectively, ω is the angular frequency, c is the speed of light and Q is the ratio of the frequency to the resonance half-intensity width. By squaring equation (1) and integrating over all frequencies, we obtain for the mean squared strain associated with a given mode:

$$\overline{\varepsilon(t)^2} \approx \frac{4c^4 Q}{\pi^2 \omega^3} R^2_{iojo}(\omega) = \frac{60GQ}{c^3 \omega} t_{or}(\omega) \qquad (2)$$

In equation (2), $R^2_{iojo}(\omega)$ is the power spectrum of the Riemann tensor, G is the constant of gravitation and $t_{or}(\omega)$ is the power spectrum of the incident gravitational energy flux, assuming the radiators are at rest relative to the Earth.

The results obtained by the Seismological Laboratory and the upper bound to the Riemann tensor and energy flux power spectra are given in Table 1 for a number of frequencies. The strain data were smoothed since the fluctuations observed were random and not correlated with the modes of the Earth.

Table 1

Fundamental mode	Period (min.)	Q (est.)	Strain² (av.)	$R^2_{iojo}(\omega)$ $\left[\frac{1}{\text{cm.}^4\ (\text{rad./sec.})}\right]$	$t_{or}(\omega)$ $\left[\frac{\text{watts}}{\text{cm.}^2\ (\text{rad./sec.})}\right]$
S_2	54·0	400	80×10^{-25}	$< 0·5 \times 10^{-75}$	< 20
S_4	25·8	350	20	2	20
S_6	16·0	300	8	8	10
S_8	11·81	250	4	5	10
S_{10}	9·66	210	2·5	7	10
S_{14}	7·47	180	1·2	10	10
S_{20}	5·78	160	1	20	10
S_{30}	4·37	120	0·6	30	10
S_{38}	3·66	100	0·6	60	10

This table indicates that the Riemann tensor power spectrum of the incident gravitational radiation cannot be larger than ≈ 10^{-75}/cm.^{4} (rad./sec.) in the vicinity of 1 c./hr. The figures for the energy flux are very high [20 watts/cm.^{2} (rad./sec.)], but not particularly meaningful. The energy associated with a given Riemann tensor depends on the reference frame in which it is defined. We chose the rest frame of the assumed radiator. If we had calculated in a rest frame fixed in the Earth's centre of mass, and averaged over the volume of the Earth, numbers for the energy flux about ten orders less would have been obtained. This is because the Earth is in free fall and the energy flux always vanishes at the pole of a geodesic co-ordinate system. Another assumption is that the Earth is behaving like an elastic body when driven by the Riemann tensor. If the Earth behaved like a liquid, the surface strains would be several orders less for a given Riemann tensor and the limits of the table correspondingly greater.

Earth strain data enable calculation only of the power spectrum of the Riemann tensor. Other effects, such as the fluctuation of the Earth's rotation period, enable the total flux to be estimated[1].

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A Maximum of Temperature in the Middle Ionosphere

J. FUCHS[1] has already pointed out the fact that from the change of electron-density (N) with height (h) conclusions as to the temperature in the ionosphere can be drawn. It was, however, not possible then to carry out exact calculations for two reasons: first, the electron-density was only known to be dependent on the apparent height (h'), and secondly, the process of electron loss was not known in more detail, so that Fuchs had to calculate with a recombination-coefficient constant as to space and time.

To-day these two difficulties have been overcome; some institutions have for some time been publishing detailed $N(h)$ profiles, for example, the Central Radio Propagation Laboratory (Boulder) hourly values for the station Puerto Rico. For the process of electron loss, I[2] have introduced the following equation:

$$L = \alpha_0 p^k . T^n . N^{m+2}$$

It has been possible recently to define the constants k and n and this has enabled me to determine from the $N(h)$ profiles the height dependency of scale height (H) and density. The results with regard to the observations of Puerto Rico can be summarized as follows:

(1) The scale height has during the day a maximum at a height of about 200 km. (2) The value of this maximum shows a considerably daily and yearly variation. In the early morning and late afternoon the maximum is much less noticeable than at noon. (3) The height in which this maximum occurs shows a corresponding daily and annual variation: at noon during the summer the maximum of H rests at a height of about 220 km.; it descends to about 180 km. in the morning in winter. (4) Above this maximum the scale height decreases rapidly, and I presume that the gradient dH/dh is getting small only a little below the $F2$ peak. (5) The decrease in density with height corresponds roughly to the result which Priester[3] was able to publish recently.

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GEOLOGY

Flow Folding in Rocks

PROF. L. U. DE SITTER[1] considers that in flow folding "internal movement itself is no longer orientated but can take any direction", and that the position of shear planes is random. He believes it developed most typically in incompetent rocks like salt, or at very high confining pressures or high temperatures. It is further maintained that this is the only true plastic deformation of rocks. These beliefs have recently been reiterated and enlarged on[2].

Such views appear to be becoming general, and Ball[3] further characterizes monogenetic folds formed by flow by stating that "the quartz fabric will tend to be markedly heterogeneous, and the symmetry of the fabric will not correspond to the symmetry of the fold".

A brief account of the concepts on plastic flow of rocks will indicate that the current use of the term 'flow' has probably to do with only one type, which is difficult to accept as occurring within rocks.

Flow in rocks is a permanent change of form without apparent rupture, and any fracturing that may occur is microscopic. This property of flow is referred to as plasticity[4]. Above microscopic level such plastic flow is a continuous deformation where neighbouring points in the material remain so during the deformation.

Leith[5] and others have stated that flow takes place by interior readjustments of rock substances by chemical, mineralogical, and mechanical changes. Commonly, the deformation results in a parallel arrangement of the constituents of the rock mass, producing a banded structure, schistosity or cleavage[6]. However, flow may leave no evidence in any parallel arrangement.

Little is known of the mechanics of plastic flow. Nevertheless, possible comparisons between plastic and viscous flow can be made[7], and the mechanics and the successful application of steady, stable, laminar flow to a number of folds have been described in papers by Cloos[8] and Carey[9,10].

Folds that appear to have resulted from a laminar type of flow more or less perpendicular to the rock layers are characterized by thickened and thinned beds, crenulation of bedding planes, and generally a persistence in depth. Where planar discontinuities have developed and folding has progressed by movement along these planes the resulting deformation has been termed a shear fold.

It appears that these laminar-flow folds are excluded from de Sitter's class of flow folds which, because of the postulated random movement, must refer to a mechanism comparable with turbulent viscous flow.

According to reasonable calculations magma is intruded in most dykes by means of laminar flow and turbulence is only achieved in those magmas which have low viscosity due to super-heating or to a high content of volatile material[11]. By analogy it appears most unlikely that a type of turbulent flow can ever be achieved within rocks, which have, in comparison, enormously high viscosities.

It is, therefore, suggested that the folds at present believed to belong to de Sitter's class of flow folds should be reconsidered, and attempts made to interpret them in terms of laminar flow, for not all folds that result from laminar flow may appear geometrically simple. It has been proposed that lateral laminar flow along involuted planes within liquefied layers of sand can account for convolute folding that may occur within them[12]. Is it perhaps possible that the complicated plastic folds with, at first sight, random orientation of the foliations, are in point of fact due to similar unusual patterns of laminar flow?

It appears that the class of flow folds, the present status of which is here questioned, should be redefined to include folds resulting from laminar plastic flow. In so far as all rocks flow during folding, limits must necessarily be rather arbitrary. Competent layers, which retain approximately their original thickness perpendicular to the bedding, show only sufficient flow for them to be flexed. Thus, flexural or concentric folds, and folds of patterns determined by the flexing of competent layers within an interbedded sequence of competent and incompetent horizons would be omitted. Only those folds determined by flow rather than flexure should be included. Flow folds may, therefore, be defined as contortions affecting only incompetent beds, which have thickened and thinned without the aid of ruptures above microscopic levels.

This communication is submitted by permission of the Director of the British Guiana Geological Survey.

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Basutoland Kimberlites

SINCE 1954 a prospecting programme for diamonds in the British Protectorate of Basutoland has led to the discovery of a new predominantly basaltic kimberlite province to the east of the micaceous kimberlite province in the Orange Free State of the Union of South Africa. The kimberlites are intruded into Karroo sediments and lavas and comprise both dykes and diatremes. Petrographically the kimberlites are similar to kimberlites in other parts of South Africa[1,2] and they contain inclusions of eclogite, granulite, peridotite and ilmenite in addition to inclusions of the Karroo country rocks.

Spectrographic analyses of the kimberlites reveal that the trace elements, like the major elements, can be divided into two contrasting suites: (*a*) those in amounts typical of ultrabasic rocks; (*b*) those in amounts typical of alkaline late differentiates. Like the ultrabasic potassic rocks of Uganda which exhibit similar chemical features[3,4], it is believed that kimberlite owes its origin to reaction between a carbonatitic magma and crustal granite. More detailed results are to be published shortly.

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PHYSICS

Normal Mode Interpretation of the Sound Propagation in Whispering Galleries

THE propagation of sound in 'whispering galleries'[1] is a very remarkable phenomenon, and in Lord Rayleigh's *Theory of Sound* he gives his well-known interpretation based on observations at St. Paul's Cathedral in London. According to my observation, which agrees with Lord Rayleigh's comment in his book, the opinion of the Astronomer Royal ascribing the phenomenon to the reflexion at the upper dome is hard to accept. This follows because at the diametrically opposite point of the gallery the whisper can scarcely be heard, whereas it becomes stronger as one approaches the speaker.

Lord Rayleigh's explanation is, to use present-day terminology of elastic surface waves, based on 'ray theory'. There is, however, another way of describing so-called 'normal mode theory', and the following analysis based on this theory is in accord with the ray theory interpretation given by Lord Rayleigh.

Adopting the velocity potential ϕ, we have a solution referred to polar co-ordinates R, θ and φ as follows:

$$\phi = ([p/c]R)^{-1/2} J_{n+1/2}([p/c]R) Y_n(\theta,\varphi)\exp(ipt) \quad (1)$$

where p is the frequency, c the velocity of sound, J_ν is the Bessel function and $Y_n(\theta,\varphi)$ the spherical surface harmonics. Assuming that the wall is approximately rigid, the boundary condition, a, being the radius of the gallery, is:

$$\text{at } R = a; \quad \partial\phi/\partial R = 0 \quad (2)$$

which gives an equation of the form:

$$\partial[\xi^{-1/2} J_{n+1/2}(\xi)]/\partial\xi = 0, \; \xi = (p/c)a \quad (3)$$

Since the circumference of the gallery is roughly 100 yards, and the wave-length of the voice is about 1 yard, n is about 100.

Trying the numerical solution for smaller values of n, we find that $n + 1/2 < \xi$. Hence we employ the next form of asymptotic formula for the Bessel function with large orders[2]:

$$J_\nu(\nu\sec\beta) \sim \left(\frac{2}{\pi\nu\tan\beta}\right)^{1/2}\left[\cos Q + \frac{1}{\nu}\cot\beta . \left(\frac{1}{8} + \frac{5}{24}\cot^2\beta\right)\sin Q\right] \quad (4)$$

$$Q = \nu(\tan\beta - \beta) - \pi/4$$

and put it into the above expression. Then we have the characteristic equation:

$$\frac{1}{\nu}\left(\frac{7}{24}\tau^3 + \frac{7}{8}\tau\right) + \left[\frac{1}{\nu^2}\left(\frac{35}{48}\tau^6 + \frac{35}{24}\tau^4 + \frac{9}{8}\tau^2\right) + 1\right] . \tan Q = 0 \quad (5)$$

$$\tau = \cot\beta$$

$$\nu = n + 1/2 \text{ and } \nu\sec\beta = \xi \quad (6)$$

The approximate solution of this equation is obtained assuming that $\nu\beta^3$ tends to a certain value different from zero when ν increases indefinitely, namely:

$$\frac{7}{24}\frac{1}{\nu\beta^3} + \left(\frac{35}{48}\frac{1}{\nu^2\beta^6} + 1\right) . \tan\left(\frac{1}{3}\nu\beta^3 - \frac{\pi}{4}\right) = 0 \quad (7)$$

The root is

$$\nu\beta^3 = 1{\cdot}99 \quad (8)$$

which, combined with (6), gives:

$$\xi = (n + 0{\cdot}5) + 0{\cdot}79(n + 0{\cdot}5)^{1/2} \quad (9)$$

The intensity distribution of ϕ with regard to radial direction is given by the function:

$$\Phi = A . ([R/a] . \xi)^{-1/2} J_{n+1/2}([R/a] . \xi) \quad (10)$$

in which n and ξ are connected by the relation (9). This function can be evaluated by the above asymptotic expansion (4) and other similar formulæ.

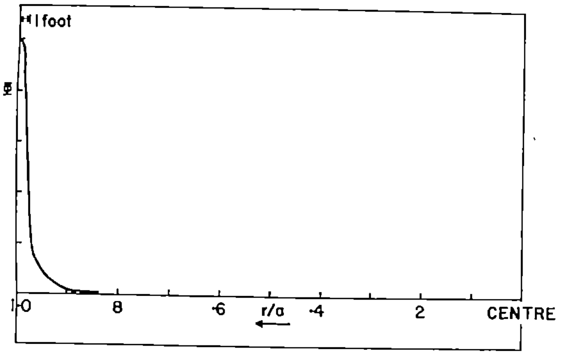

Fig. 1

Assuming $n = 100$, I carried out the numerical computation of the function Φ, in which A was given a value such that the function becomes unity at the surface $R = a$.

The property of the curve given in Fig. 1 agrees well with my auditory observation in St. Paul's Cathedral. If the ear is moved about 10 in. from the wall the intensity of the sound does not change much; but after that it falls off rapidly. Since the circular corridor along the wall is about 6 ft. wide, the observation cannot be continued further than that; but in fact the whisper is scarcely audible at the edge of the corridor.

These characteristics are exactly those of the so-called guided waves. In a homogeneous medium of half-space, sound waves do not give surface waves like Rayleigh waves in a solid. However, if the medium is surrounded by a rigid spherical wall, a kind of guided wave can exist, and this is what is observed in whispering galleries. The surrounding wall is neither exactly spherical nor circular cylindrical in the case of St. Paul's Cathedral; but these calculations give a rough approximation of what takes place in the gallery.

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Measurement of Pressure in Vacuum Physics

THE torr has recently been adopted as the British standard unit for the measurement of pressure in vacuum physics; but the fact that proposals have been made for further change[1,2] indicates that the present system is not yet satisfactory. The recent proposals have been concerned with definition of units; but the practical expression of pressures in terms of these units appears also to be due for revision.

The range of pressures encountered in vacuum physics is at present about 10^3–10^{-11} torr, so that to cover this range with a single unit the conventional method is to use the exponent; the alternative method of expression of pressures as small decimal fractions of a torr is obsolescent, and its main continued use appears to be in the usual calibration of the scale of the McLeod gauge. Thus we are at present expressing pressures less than atmospheric with a range of exponents which may be positive or negative, with the zero exponent corresponding to no clearly recognizable pressure. As improvements in high-vacuum techniques continually decrease the pressures attainable in vacuum systems the problem of expression becomes aggravated as we are forced to use larger negative exponents; this makes more obvious the terminological inconsistency of a 'higher' vacuum corresponding to a smaller number.

A similar problem, the expression of hydrogen-ion concentration in aqueous solution, was elegantly solved by the introduction of the pH scale, which has been universally adopted and has proved satisfactory over many years of use. In most vacuum measurements the problem is quite analogous; we are concerned mainly with the order of magnitude of the pressure.

It is proposed therefore that the new unit the vac[2], which has excellent theoretical justification, would be an ideal basis for a p Vac scale. If zero on the scale is taken as 10^3 vac, which is almost one standard atmosphere, it is possible to define a scale analogous to the pH scale:

$$p\,\text{Vac} = -\log \text{vac} + 3$$

Thus, zero is atmospheric pressure, all practical vacua will be positive, and the larger p Vac, the higher the vacuum.

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The Special Theory of Electromagnetism

THE law of addition of collinear velocities as derived from the Lorentz transformation in special relativity is:

$$W = \frac{U + V}{1 + UV/c^2} \tag{1}$$

U and V being the components, W the resultant velocity and c the velocity of light. It follows that W can never be greater than c.

Despite this failure in kinematics of the classical law of vector addition, that law is still used in other fields of physics. This communication shows what modifications to electrostatic theory are necessary if a rule analogous to equation (1) is adopted in its place.

Introducing 'electric interval' $d\sigma$ by the relation:

$$d\sigma^2 = dr^2 - \varkappa^{-2}d\varphi^2 \tag{2}$$

where dr and $d\varphi$ have their usual meaning for an observer and $\varkappa$ is a universal constant, the addition of collinear electric vectors now follows the rule:

$$E = \frac{E_1 + E_2}{1 + E_1E_2/\varkappa^2} \tag{3}$$

and $\varkappa$ is recognized as the limiting attainable resultant, presumably corresponding to a break-down field-strength.

Discrepancies from classical theory will be imperceptible in weak fields, so that support for the new hypothesis must be sought under conditions near breakdown.

Considering the inverse square law, a geometrical concept in which the possible effect of the field on measurement of distance is ignored, it appears that distance must be replaced by interval in its formulation, so that the field of an electron is represented by :

$$\frac{d\varphi}{d\sigma} = -\frac{e}{\sigma^2} \qquad (4)$$

e being the charge. The measured field-strength is then :

$$\frac{d\varphi}{dr} = -e(\sigma^2 + e^2/x^2)^{-1/2} \qquad (5)$$

which compares with the Born and Infeld formula.

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CRYSTALLOGRAPHY

Out-of-Focus Electron Microscope Images of Edges of Crystal Lattices

IN a recent communication, Hashimoto and Watanabe[1] report the observation of extra lines outside the geometric bounds of the electron microscope image of a crystal of copper phthalocyanine when the microscope is de-focused to obtain the Fourier image of order $N = \frac{1}{2}$ (see Cowley and Moodie[2]). They state that "such lines are not explicable by the theory of Cowley and Moodie"

The paper of ours[2] to which they refer contains not only a discussion of such effects as essential components of the concept of Fourier images but also a sequence of out-of-focus images obtained from an amplitude object with visible light which clearly demonstrates that such effects occur. The periodic object in our case was interrupted by a small internal non-periodic region, rather than by a sharp external boundary as in the case quoted by Hashimoto and Watanabe ; but the progressive extension of the periodic grating image across the boundary as the order number of the Fourier image increases is clearly visible. Their electron micrographs may, in fact, be considered as further evidence in favour of our contentions at that time : they demonstrate that Fourier image phenomena are present and important in the electron microscope imaging of crystal lattices.

It appears that Hashimoto and Watanabe have considered not our theory of Fourier images but the simplification of it represented by their Fig. 2 and associated discussion, which ignores Fresnel diffraction effects and cannot, therefore, give an adequate account of the observation under discussion. This limitation should not, however, be attributed to our theory.

The Fourier image theory which we presented in our initial paper[2] was developed in detail only for periodic objects. It may be applied equally well to non-periodic objects. In either case, however, the theory is more conveniently developed on the basis of our reformulation of physical optics theory presented in later papers[3,4]. If we assume the incident radiation to be coherent and plane-parallel, the complex amplitude of the wave in an image out-of-focus by an axial distance, R, is given by :

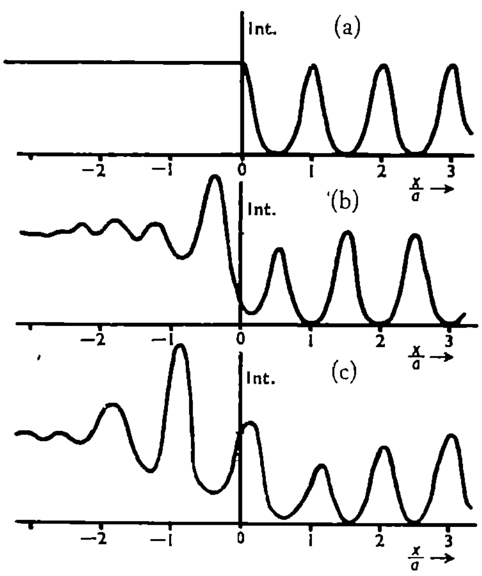

Fig. 1. The calculated intensity distribution at the edge of a periodic amplitude object of the form $\frac{1}{2} + \frac{1}{2}\cos(2\pi x/a)$, (*a*) in focus image (Fourier image of order $N = 0$), (*b*) out-of-focus (Fourier image of order $N = \frac{1}{2}$), and (*c*) Fourier image of order $N = 1$

$$\psi(x,y) = q(x,y) \star \exp\{-ik(x^2 + y^2)/2R\} = \iint q(X,Y)\exp\{-ik[(x-X)^2 + (y-Y)^2]/2R\}\mathrm{d}X.\mathrm{d}Y$$

where $q(x,y)$ is the transmission function of the object. The intensity distribution is then given by $I(x,y) = \psi(x,y).\psi^*(x,y)$.

For the particular case of a crystal edge considered by Hashimoto and Watanabe, we may put :

$$q(x,y) = q_1(x,y).S(x) + S(-x)$$

where $q_1(x,y)$ is the periodic transmission function for an infinite, periodic crystal lattice and $S(x)$ is the step-function :

$$S(x) = \begin{cases} 0 \text{ for } -\infty < x < 0 \\ 1 \text{ for } 0 < x < \infty \end{cases}$$

If we put $q_1(x,y) = \frac{1}{2} + \frac{1}{2}\cos(2\pi x/a)$, which corresponds to an amplitude grating similar to that used by Hashimoto and Watanabe as an optical analogue, the calculated intensity distributions are as shown in Figs. 1*a*, *b* and *c* for the Fourier images of order $N = 0$, $N = \frac{1}{2}$ (that is, $R = a^2/\lambda$) and $N = 1$ (that is, $R = 2a^2/\lambda$). Figs. 1*a* and *b* clearly reproduce the relevant features of the intensity distributions, including the one relatively strong and two weak lines beyond the edge of the grating, visible in their Figs. 3*a* and *b* (ref. 1).

In the same way we may calculate the intensity distribution to be expected at the boundary in an out-of-focus image of a copper phthalocyanine crystal if we put :

$$q_1(x,y) = \exp\{i\sigma\varphi(x,y)\}$$

where $\varphi(x,y)$ is the periodic function representing the projection of the potential distribution in the crystal lattice and σ is a constant. It has been established that, to a first approximation, thin crystals act as phase gratings for electrons[5] and a study has been made of the contrast in Fourier images of such gratings[6]. We hope to publish elsewhere a more detailed account of the images of crystal lattices formed in electron microscopes.

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CHEMICAL ENGINEERING

A Rapid Method for the Determination of Tetramethyl Lead and Tetraethyl Lead in Petrol

THE use of tetramethyl lead as anti-knock additive in petrol sets a new problem to the petroleum laboratories in connexion with the determination of these additives. The standard A.S.T.M. method *D* 526–56 cannot be used for the analysis of petrols containing a mixture of tetramethyl lead and tetraethyl lead or for the determination of lead in a sample containing alkylated lead additives.

We have tried to work out a rapid method for the qualitative determination of tetramethyl and tetraethyl lead in petrols. First, we tried to use a colour reaction with dithizone reagent. Dithizone in chloroform solution is added to the petrol. The mixture is shaken with water and a red colour appears in the organic phase. The reaction mechanism is apparently different for the tetramethyl and the tetraethyl lead. Tetraethyl lead seems to be less stable than tetramethyl lead; it hydrolyses more quickly and a red colour appears, whereas tetramethyl lead does not undergo any reaction. This test being extremely sensitive (limit $0{\cdot}04\gamma$), errors due to impurities in glass or water are likely to occur. It is also unsuitable for use with a red-coloured petrol.

We therefore tried another method, using petrol fractions obtained by distillation. Afterwards, we examined the different fractions by means of a Zeiss flame spectrophotometer *M4Q* at 406 mμ in order to determine the lead.

Generally, we have taken four fractions and the residue: initial boiling point to 100° C.; 100° to 125° C. (fraction containing the tetramethyl lead); 125° to 150° C.; 150° to 175° C.; and the residue (containing the tetraethyl lead). About 250 c.c. of petrol are distilled on a 'Vigreux' column (30 cm. in length), in 2 hr. We observed that the slower the distillation, the better the separation of the lead in the spectrophotometric determination.

To check the method, we used a platformate containing tetramethyl lead, a platformate containing tetraethyl lead and a platformate containing tetramethyl and tetraethyl lead. The figures in Table 1 indicate the galvanometer readings expressing the light intensity of the flame. The apparatus was standardized at a reading of 50 with a platformate containing 4·2 c.c. tetramethyl lead per Imperial gall. An oxy-acetylene flame is used. Only the figures in the horizontal lines of the table should be compared with each other. In this determination we did not consider the flame background of the different fractions. Since these results on lead distribution in known mixtures were reasonable, we examined commercial samples. The results are summarized in Table 2.

The petrols showing high readings in the 100° to 125° C. fraction were those giving aberrant results in the *A*.S.T.M. determination *D* 526–56. Moreover, these results tally with those advertised by petroleum companies. The method given here is simple and allows one to estimate tetramethyl and tetraethyl lead. Anti-knock quantitative analysis may be carried out in the usual way.

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Table 1

Fractions	Initial boiling point to 100° C.	100° to 125° C.	125° to 150° C.	150° to 175° C.	Residue
Platformate + tetramethyl lead	5	53·5	26	10	3
Platformate + tetraethyl lead	5	9·5	10	14	28
Platformate + tetramethyl lead + tetraethyl lead	18	33	10	21	37

Table 2

Fractions	Initial boiling point to 100° C.	100°–125° C. (tetramethyl lead)	125°–150° C.	150°–175° C.	Residue (tetraethyl lead)
Sample *A*, 'super'	4	47	30	20	7
Sample *B*, 'super'	7	41	37	25	14
Sample *C*, 'super'	9	44	36	19	17
Sample *D*, 'super'	8	38	25	—	8
Sample *E*, 'normal'	3	11	22	—	60
Sample *F*, 'super'	5	13	17	27	56
Sample *G*, 'normal'	4	6	8	14	69
Sample *H*, 'super'	3	12	24	24	50

CHEMISTRY

Influence of Chemical Isomerism on the Szilard–Chalmers Processes in Solids

THE recent appearance[1] of a communication concerning the Szilard–Chalmers effect in *cis*- and *trans*- dichloro- *bis* (ethylenediamine) cobalt (III) nitrate prompts us to report the following results.

A considerable amount of work has been published in which processes associated to the Szilard–Chalmers effect are studied in solid-state complex combinations.

Less attention, however, has been given to the influence of chemical isomerism. The question arises whether in two chemical isomers the annealing of reactor irradiation damage occurs in the same way.

Zuber[2] was the first to study the Szilard–Chalmers effect in crystalline D-[Co en$_3$](NO$_3$)$_3$. After irradiation of the dextro complex and the addition of lævo isomer as carrier, he found by resolving [Co en$_3$](NO$_3$)$_3$ that, of 5 per cent annealed atoms after irradiation, only 4·5 per cent annealed as the dextro, and 0·5 per cent annealed as the lævo, isomer. It would be interesting to examine also the irradiated lævo isomer and to compare the behaviour of the two isomers. Recently, Saito and co-workers[3], investigating the behaviour of hot bromine atoms in cobalt complex salts, found no noteworthy differences in the yield of complexed radiobromine for *cis–trans* isomers. They did not examine the retention of radiocobalt.

Our attempts to find evidence of differences in the behaviour of chemical isomers in radiocobalt annealing processes were more successful. Cobalt complex combinations exhibiting geometrical, optical, hydration, ionization, or linkage isomerism were irradiated for 40 hr. in the *IFA* reactor. The samples were irradiated in aluminium ampoules in air. The irradiation conditions were as follows: thermal neutron flux, $6 \cdot 0 \times 10^{11} n$. cm.$^{-2}$ sec.$^{-1}$; fast neutron flux, $1 \cdot 75 \times 10^{11}$ n. cm.$^{-2}$ sec.$^{-1}$; gamma dose-rate, $1 \cdot 31 \times 10^{7}$ r. hr.$^{-1}$.

The fraction annealed after irradiation was determined via retention, using a well-known analytical technique for the separation of recoiled radiocobalt[4,5]. The results are given in Table 1, together with the difference in retention for a pair of isomers.

These investigations are continuing, but some conclusions may already be drawn. It appears that chemical isomerism plays an important part in the recombination process of recoil fragments occurring simultaneously with irradiation. This would be expected also when taking account of the influence of the steric factor on the annealing process. The difference in the spatial arrangement of groups corresponding to the same general formula certainly influences the interatomic distances and the internal field of the crystal. In addition, the influence of chemical groups which may act differently depending on their function in the molecule must be considered.

The greatest differences are observed with the hydration isomers, where the water of hydration has at first sight a reducing effect on the recoiled radiocobalt, a view which has been thought to apply to permanganate salts[6]. It is only known that water molecules behave differently when co-ordinated to cobalt. The geometrical isomers exhibit also considerably different retentions. It was observed that the *cis*-position is more advantageous, the annealing being lower for the *trans*-position where steric factors cause serious hindrance. For ionization and linkage isomers it was found that the chemical bond has some influence. The differences in the retention of lævo- and dextro-combinations though smaller are present. Here also the steric factor plays a part, although the differences do not act in the same direction.

Table 1. RETENTIONS OF CHEMICAL ISOMERS AFTER REACTOR IRRADIATION

Isomerism	Chemical formula	Retention (R per cent)	Difference in retention (ΔR per cent)
Hydration	[Co(NH$_3$)$_4$Cl(OH$_2$)]Cl$_2$	68·2	39·0
	[Co(NH$_3$)$_4$Cl$_2$]Cl.H$_2$O	29·2	
Geometrical	*cis*-[Co en$_2$Cl$_2$]Cl	79·8	15·2
	trans-[Co en$_2$Cl$_2$]Cl	63·7	
	cis-[Co(NH$_3$)$_4$(NO$_2$)$_2$]NO$_3$	86·6	10·8
	trans-[Co(NH$_3$)$_4$(NO$_2$)$_2$]NO$_3$	75·8	
Linkage	[Co(NH$_3$)$_5$NO$_2$]Cl$_2$	71·1	6·8
	[Co(NH$_3$)$_5$ONO]Cl$_2$	77·9	
Ionization	[Co(NH$_3$)$_5$SO$_4$]Br	75·0	5·5
	[Co(NH$_3$)$_5$Br]SO$_4$	69·5	
Optical	D-[Co en$_3$](NO$_3$)$_3$	71·9	1·7
	L-[Co en$_3$](NO$_3$)$_3$	73·6	
	D-[Co en$_3$]Br$_3$.2H$_2$O	89·9	6·4
	L-[Co en$_3$]Br$_3$.2H$_2$O	83·5	

So far only qualitative investigations have been discussed. A systematic investigation involving analysis of recombination products and kinetic measurements is in progress and the results will be reported in due course.

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Interactions of NO_2–N_2O_4 and Niobium Dioxide (NbO_2)

THE interaction of NO$_2$ and niobium dioxide (NbO$_2$) was studied as part of a general programme to investigate the oxidation chemistry of NO$_2$ with respect to its effect upon potentially non-stoichiometric oxides. Other oxides which have been investigated have shown that NO$_2$ may act as an oxidizing agent as well as a nitrating and nitriting agent. In addition, significant quantities of physically and chemisorbed NO$_2$ are always encountered in this type of interaction[1]. In a brief introductory study carried out on niobium oxide a number of significant points were uncovered which expand the present investigations.

Niobium dioxide was prepared by reduction in a stream of dry hydrogen at 950° C. The NO$_2$ was obtained from the Matheson Chemical Co. It was transferred to the vacuum line through a magnesium perchlorate trap, and was fractionated until the solid condensate was white. At all temperatures in the range of 100–500° C. and pressure of NO$_2$ of 0·014–65 cm. mercury the oxidation of the dioxide proceeded at a rapid rate, such that the composition of Nb$_2$O$_5$ was established nearly instantly. In many cases the dioxide acted as pyrophor, igniting and immediately producing the penta oxide. For the lower pressures and lower temperatures the oxidation proceeded more slowly and was noted to take several minutes for completion. In general, the rapidity of the reaction made a study of the kinetics impossible.

Analysis of the reacted product indicated that the composition of the final product was in all cases within 0·02 stoichiometric unit of the composition NbO$_{2\cdot5}$. This was further confirmed by observing the optical reflectance in the ultra-violet. In addition

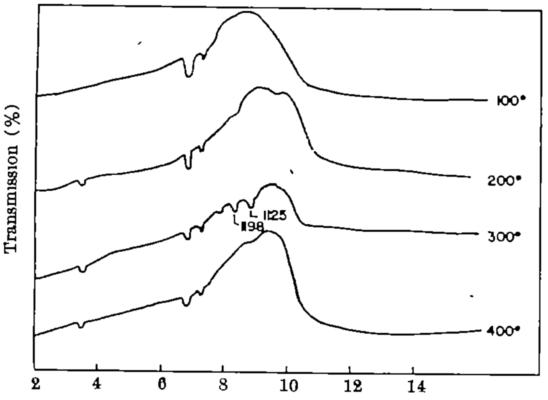

Fig. 1. Infra-red spectrum of the interaction product of niobium dioxide and nitrogen dioxide for various temperatures of interaction at a pressure of 65 cm. nitrogen dioxide

to the above stoichiometric ratio it was noted that a small amount of nitrogen was also incorporated in the oxide. At a pressure of 26·5 cm. mercury of NO_2 and in the temperature interval from 100 to 500° C. the stoichiometric ratio of nitrogen–niobium varied linearily with increasing temperature from 0·019 to 0·043 and at a $P_{NO_2} = 0·014$ cm. mercury the nitrogen content was within the limit of error of the method[2].

In an attempt to determine the origin of this incorporated nitrogen the infra-red spectra of the reacted material was obtained. This was done on a Perkin Elmer model 221 double-beam spectrophotometer. The spectra are partially reproduced in Fig. 1 for the interaction of NO_2 at 65·5 cm./mercury and Nb_2O_4 in the temperature-range of 100–500° C. Absorption peaks may be seen at ~ 3·5, 6·8, and 7·3μ due to interference from the 'Nujol' mulling agent. Secondly, a broad band is in evidence which begins at about 10·5μ. This is most probably due to the Nb–O stretching frequency[3,4]. Finally, bands may be seen at ~ 7·8, 8·3, 8 8 and 9·6μ. It is felt that the bands at 7·8 and 9·6 are probably due to the presence of some form of ionic nitrate[5] as these bands have been found to be characteristic of ionic nitrates in general. The bands at 8·3 and 8·8 appear to be due to the NO_2 group, in the form of strongly chemisorbed nitrogen dioxide or as the nitrite group[6]. It should be noted that in both these cases the presence of these species occurs at an extremely low concentration and thus does not demonstrate the inherent basic character in niobium dioxide.

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Dissociation Energy of Aluminium Monobromide

IN a recent review[1] of the dissociation energies of the gaseous monohalides of boron, aluminium, gallium and thallium, it was concluded that the best value for D_0'' (aluminium monobromide) is 105 k.cal. mole^{-1}. Dr. P. Gross has now kindly directed my attention to the work of Semenkovich[2], who has determined the standard heat of formation of gaseous aluminium monobromide by a thermochemical method similar to that used by Gross, Campbell, Kent and Levi[3] for aluminium monofluoride and monochloride. Semenkovich obtains $Q_f(\text{AlBr}_g) = +1·1$ k.cal. mole^{-1}. With $\Delta_s H(\text{Al}) = 77·4$, $Q_f(\text{Br}_g) = 26·71$, and $D_{298}'' - D_0'' = 0\ 85$, $D_0''(\text{AlBr}) = 102\ 2$ k.cal mole^{-1}. As with AlF and AlCl, this value is a little lower than two of the spectroscopic estimates from the state $A\,^1\Pi$, which, for AlBr, are 106·9 k.cal. by extrapolation, and >104·4 k.cal. from the highest observed vibrational level: it is, however, consistent with a predissociation limit which leads to $D_0'' \leqslant 105·7$ k cal.

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Triplet Bands of Carbon Monoxide: the System $e\,^3\Sigma^- - a\,^3\Pi$

SOME years ago, in a study of the triplet system, $d(?^3\Pi) - a^3\Pi_r$ of carbon monoxide, Herman and Herman[1] observed a progression of bands of similar appearance which they assigned to a new system. They suggested that the upper state of this system was d and that the lower state was a new triplet-level, lying close to $a^3\Pi$. Later work[2,3] on the forbidden systems of carbon monoxide observed in the absorption spectrum in the Schumann region has provided detailed information about some of the triplet-levels, and the object of this communication is to show that the Herman system arises in fact from the transition $e\,^3\Sigma^- - a\,^3\Pi_r$.

In Table 1, the second column gives values of band-origins of the $e - X$ system[4]. Positions of the band-heads of the Herman system[1] are given in columns three and four. The results of subtracting column four from two are given in column five. These figures are as constant as could be expected, and indicate that the bands form a progression involving the levels $v' = 2$ to 7 of $e\,^3\Sigma^-$. The mean value for the height of the lower level above $v = 0$ in the ground-state $X\,^1\Sigma^+$ is 48,520·$_5$ cm.$^{-1}$.

Table 1

v	$e-X$	$e-a$		$a-X$
	ν_0	λ	ν	ν
7	71,926 5	4,270 8	23,408 3	48,518 2
6	70,966·2*	4,454·5	22,442·9	523 3
5	69,986 4	4,657 2*	21,465 9*	—
4	68,987 9*	4,885 0	20,465 1	522 8
3	67,970 3	5,140 3	19,448 7	521 6
2	66,933 3	5,428 3	18,416 9	516 4

* Interpolated value.

The measurements of band-heads given in Table 1 refer to the longest wave-length heads of groups of three. For the $v' = 2$ and $v' = 3$ bands, further measurements are available[4]:

5428·3 Å.	18,416·9 cm.$^{-1}$		5140·3 Å.	19,448·7 cm.$^{-1}$	
		46·9			46·3
14·5	463·8		28·1	495·0	
		41·0			45·3
02·5	504·8		16·2	540·3	

The mean separation between adjacent heads is thus about 45 cm.$^{-1}$ and the origin of the lower state may be estimated to lie at 48,520·$_5$ − 45 = 48,475·$_5$ cm.$^{-1}$ above $X\ ^1\Sigma^+$. This estimate is in excellent agreement with the value of ν_{00} for $a^3\Pi$ -- $X^1\Sigma^+$ obtained from the rotational analysis[5] of the Cameron bands, namely, 48,473·9$_7$ cm.$^{-1}$.

It seems, therefore, quite certain that the Herman bands form the $v' - 0$ progression in the transition $e\ ^3\Sigma^- - a\ ^3\Pi$. No doubt other bands of this system remain to be found at longer wave-lengths.

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Identification of the Radical $\cdot CH_2(CO_2H)$

IN recent years a number of free radicals trapped in irradiated solids have been identified from their electron-spin resonance spectra. In particular, radicals of the form $XH\dot{C}CO_2H$ or $XH\dot{C}CO_2^-$ are known with $X = CH_3$ (ref. 1), NH_3^+ (ref. 2), OH (ref. 3), CO_2H (ref. 4), Cl (Pooley, D., and Whiffen, D. H., unpublished work), CH_2CO_2H (ref. 5), and $CH_2.CH_2CO_2H$ (unpublished work by A. H., J. R. M. and D. H. W.). Hitherto, however, the parent radical $\cdot CH_2(CO_2H)$ has remained unidentified. This radical has now been identified in γ-irradiated malonic acid, $CH_2(CO_2H)_2$. McConnell *et al.*[4] have fully investigated the radical $\dot{C}H(CO_2H)_2$ which is also formed when malonic acid is γ-irradiated; they reported that lines not due to $\cdot CH(CO_2H)_2$ appeared in the spectra of freshly irradiated crystals, and could be removed by heating the crystals to 50° C. for 24 hr.

A detailed study of the spectrum of irradiated malonic acid was undertaken using an electron resonance spectrometer operating at 9,000 Mc./s. A single crystal of malonic acid was γ-irradiated at liquid-nitrogen temperature. Its room-temperature spectrum indicated that at least three radicals were present. For a general orientation of the crystal with respect to the magnetic field, all three radicals have different *g* values, and each has a spectrum symmetrical about its own centre. If the hyperfine structure of the spectrum of each radical can be analysed, it is possible to identify the radical. For some crystal orientations, however, exact analysis is difficult because of overlapping of the spectra of different radicals. The spectrum of a freshly irradiated single crystal of malonic acid consisted of two exceedingly strong and narrow lines due[4] to the radical $\cdot CH(CO_2H)_2$. In addition, we observed four sharp lines of equal intensity approximately one-fifth as strong as the spectrum of $\cdot CH(CO_2H)_2$. A third radical was also present, but its spectrum was still weaker and the radical has not yet been positively identified.

The spectrum of intermediate intensity indicates the presence of a radical in which there is hyperfine interaction with two hydrogen nuclei. This spectrum could be analysed for a sufficient number of orientations to obtain a complete coupling tensor for each hydrogen atom. However, the precision is less than that which would be attainable if this were the only radical present. The principal values of the coupling tensor of one hydrogen atom were −91, −56 and −30 Mc./s., and for the other −92, −59 and −37 Mc./s. These values are essentially those to be expected for a hydrogen atom attached directly to the free radical centre[3,4,6]. They have therefore been given a negative sign, although this is not obtained directly from the spectra. The coupling tensors identify the radical as $\cdot CH_2(CO_2H)$, since no other radical with two hydrogens on the free-radical carbon atom is anticipated from the irradiation of malonic acid. The slight differences between the two tensors are less than the probable experimental error (±4 Mc./s.), although the two hydrogen atoms are not in crystallographically equivalent positions in the trapping site, and their coupling tensors need not, therefore, be absolutely identical. It appears that the methylene group ($\text{—}\dot{C}H_2$) in the radical is not rotating about the C—C bond, since free rotation would lead to a spectrum having three hyperfine lines with intensity ratios 1 : 2 : 1 for all crystal orientations.

It is expected[2,6] that the least negative, intermediate, and most negative principal values of the coupling tensor will occur for directions parallel to the C—H bond, perpendicular to the plane of the radical, and in the plane of the radical but perpendicular to the C—H bond, respectively. The intermediate axes for both tensors therefore should be parallel to each other. This was found to be the case, the experimental value for the angle between the intermediate axes of the two tensors being 4° with an error of approximately 5°. The major and minor axes of each tensor should intersect at an angle equal to the HCH angle. This angle was calculated from the tensors to be 116 ± 5°. In a planar radical, this angle would be 120° for strict sp^2 hybridization, although van der Waals repulsion between the hydrogen atoms and the carboxylic acid group might give rise to a slight reduction in this value.

The electron-resonance spectra of irradiated malonic acid thus support a planar structure for the substituted methyl radical, in agreement with previous work[7] on the methyl radical itself.

This investigation was undertaken as part of the programme of the Basic Physics Division of the National Physical Laboratory, and will be published in detail elsewhere. This report is published by permission of the Director.

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BIOCHEMISTRY

Fatty Acid Specificity for the Esterification of Vitamin A and Cholesterol by Intestinal and Pancreatic Enzymes in Rats

Vitamin A and cholesterol esters have been shown to undergo extensive hydrolysis in the lumen of the small intestine during the process of absorption; they are re-esterified to appear in the lymph mostly as esters[1,2]. However, the vitamin A esters of the lymph, blood and liver of the rat are formed by long-chain fatty acids[3] and in the normal rat liver, probably as palmitates[4]. On the other hand, cholesterol esters are usually made up of poly-unsaturated fatty acids in the lymph and blood of rats[5]. For the absorption of the two lipid materials, the enzymes of the pancreas have been largely implicated, while not much attention has been paid to the possible role of the mucosal enzymes. From the behaviour of the mucosal enzymes, as presented here, it appears that probably these enzymes play a more important part in the re-esterification of the two lipid materials during their absorption.

Acetone-dried powder was prepared from the intestinal mucosæ and pancreas of normal stock rats of this Institute by grinding the tissues in 10 vol. of acetone pre-cooled to −15°. The powder was suspended in cold distilled water in the proportion of 1 gm. powder per 10 ml. water for 1 hr. and was then centrifuged for 10 min. at 10,000*g* in the cold. The clear supernatant liquid was suitably diluted and used as the source of the enzymes. The reaction mixtures for cholesterol esterification contained 5 μmoles of free cholesterol and 10 μmoles of the given fatty acid in 0·5 ml. of ethanol, 3 ml. of Michaelis veronal buffer (0·1 *M*), *p*H 6·1, 25 mgm. of sodium taurocholate and the enzyme extracts representing 10 mgm. of pancreatic or 25 mgm. of intestinal powder. The final volume was made up to 5 ml. and incubation was for 30 min. for pancreas and 60 min. for intestine at 37°. The reaction mixtures for vitamin A esterification contained 1 μmole of vitamin A alcohol and 2 μmoles of the given fatty acids in 0·5 ml. ethanol, 3 ml. of Michaelis veronal buffer (0·1 *M*), *p*H 6·6 and the enzyme extract representing 2 mgm. of pancreatic or 5 mgm. of intestinal powder. The final volume was made up to 5 ml. and both were incubated for 30 min. at 37°. The reaction mixtures were extracted twice with light petroleum ether (40–60°) after stopping the reactions by the addition of 5 ml. of ethanol. We have been using chromatographic procedures for the separation of vitamin A ester and alcohol, and we have found that the same method can be used for the quantitative separation of free and esterified cholesterol also. The two forms of vitamin A and cholesterol were, therefore, separated on alumina columns[6] and estimated as described previously[7].

Results summarized in Table 1 clearly show that both pancreatic and intestinal enzymes esterify cholesterol preferentially with unsaturated fatty acids. Our findings with the pancreatic enzymes agree broadly with the earlier reports of Hernandez and Chaikoff[8] and Swell *et al.*[9], who have shown that the presence of unsaturation in the fatty acid is necessary for the effective esterification of cholesterol by the pancreatic enzymes. However, according to Hernandez and Chaikoff[8], while oleic and linoleic acids showed almost the same rates of esterification, linolenic acid was only 13 per cent as active as oleic acid, and our results with the pancreatic enzymes show linoleic and linolenic acids to be 68 and 40 per cent as efficient as oleic acid. In contrast, as demonstrated here, the mucosal enzymes exhibit progressively increased esterification with increase in the unsaturation of the fatty acid. If the pancreatic enzymes were to re-esterify cholesterol for its transport during absorption, the oleate should be the major ester in the lymph and blood, but in these tissues the cholesterol is found esterified mostly with polyunsaturated fatty acids[10–12]. It thus seems clear that the mucosal enzymes, rather than the pancreatic enzymes, are responsible for the re-esterification of cholesterol during absorption.

Table 1. Fatty Acid Specificity for the Esterification of Vitamin A and Cholesterol by the Enzymes of Intestine and Pancreas of Rats

Fatty acid	mμmoles cholesterol esterified/mgm. protein/hr.		mμmoles vitamin A alcohol esterified/ mgm. protein/hr.	
	Pancreas	Intestine	Pancreas	Intestine
Acetic	0·0	0·0	3·0	2·3
Butyric	0·0	0·0	2·3	1·5
*Iso*valeric	0·0	0·0	2·3	2·3
Caproic	0·0	0·0	2·3	1·5
Lauric	60·0	7·5	938·0	285·0
Myristic	40·0	5·0	868·0	142·5
Palmitic	47·5	5·5	896·0	159·0
Stearic	50·0	5·7	798·0	139·5
Oleic	1,313·0	50·0	840·0	130·5
Linoleic	885·0	71·5	854·0	135·8
Linolenic	515·0	82·0	882·0	132·0

Regarding the esterification of vitamin A, neither tissue showed any preference for unsaturation, and it was effectively esterified with fatty acids containing more than 12 carbon atoms by both the tissues. It should be mentioned here that Pollard and Bieri[13] had similar experience with chick pancreas. This again would explain the presence of higher fatty acid esters of vitamin A in the blood and lymph of rats[3].

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In vitro Decarboxylation of Tryptophans by Mammalian Decarboxylase

TWENTY-TWO tryptophans have been examined in order to check the specificity of the so-called 5-hydroxytryptophan decarboxylase[1,2], and at the same time to see whether any of these compounds could be decarboxylated at a rate similar to that known for 5-hydroxytryptophan (5-HTP). The present work was intended as preliminary to further investigations on the possibility of non-biogenic tryptophans interfering in the biosynthesis, actions and metabolism of endogenous 5-hydroxytryptamine (5-HT), especially of brain 5-HT.

The tryptophans examined were the following: L-tryptophan, DL-5-hydroxytryptophan, DL-4-hydroxytryptophan (4-HTP), DL-6-hydroxytryptophan, DL-7-hydroxytryptophan, DL-2-hydroxytryptophan, DL-α-*iso*-5-hydroxytryptophan, DL-5-methyltryptophan, DL-5-methoxytryptophan, DL-5-benzyloxytryptophan, DL-5-nitrotryptophan, DL-5-aminotryptophan, DL-5-hydroxy-β-methyltryptophan, DL-5-hydroxy-N,N-dimethyltryptophan, DL-5-hydroxy-6-methoxytryptophan, DL-5-hydroxy-2-methyltryptophan, DL-5-benzyloxy-6-methoxytryptophan, DL-5-hydroxy-N-acetyltryptophan, DL-5-acetoxy-N-acetyltryptophan, DL-4-methyltryptophan, DL-4-benzyloxytryptophan, DL-6-benzyloxytryptophan.

The enzyme preparations were obtained by centrifugation of homogenates of different guinea pig, rat, mouse and rabbit tissues in a Spinco preparative centrifuge, at 15,000 r.p.m. Decarboxylation was carried out in a conventional Warburg apparatus, at 38° C. and *p*H 7·4. The flasks contained 4 m.moles of the substrate amino-acid, 50 μmgm. pyridoxal phosphate, 0·07 μmole phosphate buffer, *p*H 7·4, and 2 ml. of the enzyme preparation, corresponding to 1 gm. fresh tissue. The total volume in the flasks was 2·4 ml. Carbon dioxide produced was determined 15 min. after the addition of 0·2 ml. of *N* sulphuric acid.

Of the tryptophans examined only 5-HTP, 4-HTP, 5-hydroxy-N-acetyltryptophan and 5-acetoxy-N-acetyltryptophan were found to be decarboxylated using the above conventional, and not very sensitive, Warburg technique.

However, as clearly shown by *in vivo* studies, decarboxylation of 5-hydroxy-N-acetyltryptophan and 5-acetoxy-N-acetyltryptophan was preceded by their hydrolysis to 5-HTP. Thus, only 5-HTP and 4-HTP actually acted as substrates for the decarboxylase.

Table 1 shows the results of a 30-min. incubation of DL-5-HTP, DL-4-HTP and dopa (3,4-dihydroxyphenylalanine was used only for the sake of comparison) with enzyme preparations from various tissues.

The carbon dioxide expected for complete decarboxylation was 44·7 μl. Addition of a mixture of 4-HTP and 5-HTP to a guinea pig kidney decarboxylase preparation led to a stoichiometric liberation of carbon dioxide consistent with the decarboxylation of both substrates.

The enzymic activity of the guinea pig kidney preparation was strongly inhibited, at *p*H 6·8, by the addition of α-methyldopa (8μmoles), caffeic acid (8 μmoles) and cynarine (8 μmoles). However, inhibition of decarboxylation of 5-HTP was more complete than that of 4-HTP and dopa.

In sharp contrast to dopa, neither 5-HTP nor 4-HTP was attacked by bacterial decarboxylase (acetone-dried cells of *Streptococcus faecalis* R.).

Table 1. *In vitro* DECARBOXYLATION OF 5-HTP, 4-HTP AND DOPA BY ENZYME PREPARATIONS FROM DIFFERENT TISSUES

Enzyme preparation	Substrate	Carbon dioxide formed in 30 min. (μl.)
Guinea pig liver	5-HTP	32·0
,, ,, ,,	4-HTP	39·0
,, ,, kidney	5-HTP	42·2
,, ,, ,,	4-HTP	33·0
,, ,, ,,	Dopa	41·5
,, ,, intestine	5-HTP	37·5
,, ,, ,,	4-HTP	36·5
,, ,, ,,	Dopa	40·0
Rat intestine	5-HTP	0
,, ,,	4-HTP	0
,, ,,	Dopa	0
Mouse brain	5-HTP	12·5
,, ,,	4-HTP	20·0
Rabbit lung	5-HTP	0
,, ,,	4-HTP	0

The above experimental results show that the so-called 5-HTP-decarboxylase is not an enzyme specific to 5-HTP, and that 4-HTP may be the precursor amino-acid of the 4-hydroxyindolealkylamines (psilocin and psilocybin) occurring in the living organism. In a recent paper, Udenfriend, Lovenberg and Weissbach[3] also suggest that the existence of separate dopa and 5-HTP decarboxylase should be withdrawn.

We are indebted to Prof. D. D. Woods, Department of Biochemistry, University of Oxford, who kindly supplied samples of acetone-dried cells of *Streptococcus faecalis* R.; L-tryptophan, DL-5-methyltryptophan and DL-4-methyltryptophan were purchased from Hoffmann-La Roche, Basle; DL-α-*iso*-5-hydroxytryptophan was kindly supplied by Dr. F. Häfliger, J. R. Geigy A.G., Basle; DL-5-nitrotryptophan and DL-5-aminotryptophan were kindly supplied by Dr. G. Cavallini, Vismara Terapeutici, Milan; all the other tryptophans were synthesized in the Farmitalia Research Laboratories, Milan.

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An Evaluation of the Use of Digitonin for the Analysis of Fæcal Sterols

NUMEROUS reports have appeared in the literature dealing with the analysis of fæcal sterols[1–3]. Before the complexity of the sterol mixture of fæces was appreciated[4], a commonly employed procedure for the analysis of coprostanol was based on the difference between the total sterol determined by gravimetric analysis of the digitonide of the mixture and the amount of cholesterol or 'chromogenic sterol' calculated from the colour produced with the Liebermann–Burchard reagent[1,2]. While many investigators recognize that the chromogenic property of coprostanol will introduce an error in the analysis if coprostanol is determined as cholesterol using the digitonide precipitation procedure, a more serious error arises from the fact that the digitonide of coprostanol is significantly soluble in many of the solvent mixtures used (for example, the Sperry–Webb determination for cholesterol[5]). This unusual solubility property of coprostanol digitonide was recognized early by Dam[6] when he reported that in contrast to cholestanol digitonide, the digitonide of coprostanol was significantly soluble in methanol, a property which allowed the crude separation of the two sterols. This information prompted us to study the solubility of coprostanol digitonide in the solvent mixture routinely employed in the Sperry–Webb procedure and to compare our results with cholesterol digitonide as a reference.

Cholesterol used for the present work was obtained from Armour and Co. and purified via the dibromide (m.p. 147–8°). The coprostanol was obtained from rat fæces and was previously isolated in this laboratory[7] as a chromatographically pure zone on silicic acid/'Celite'. To eliminate the possibility of the presence of coprostan-3α-ol, the coprostan-3β-ol was further purified through the digitonide (Nutritional Biochemical Company), washed with acetone/ether (1 : 2) and ether, and regenerated as the 3β-sterol by cleavage of the digitonide with pyridine (m.p. 98–99° C.). Standard solutions of cholesterol and coprostanol were prepared in absolute ethanol/acetone (1 : 1) and suitable aliquots (300 μgm.–1,400 μgm.) were analysed in triplicate as follows: The aliquots were transferred to either a centrifuge tube or a colorimeter tube and the ethanol–acetone removed under vacuum at 50–60° C. To the samples in the centrifuge tubes, 2 or 4 ml. of absolute ethanol/acetone (1 : 1 v/v) were added. A drop of 10 per cent acetic acid was added to each tube, and either 1 ml. or 2 ml. of a 0·5 per cent solution of digitonin in 50 per cent ethanol brought the final volume to 3 or 6 ml. respectively. The mixture was heated for 15 min. in a water-bath at 50° C. and allowed to stand for 18–24 hr. In every case, the 5 or 10 mgm. of digitonin added was in excess of the theoretical requirement. The digitonides were washed and dried exactly as described by Sperry–Webb[5]. After the removal of ethanol–acetone and ether from the pooled washings, the remaining sterol was partitioned between ether and water. The ether extract was washed several times with water. The mixture was taken to dryness *in vacuo*, and the recovered sterol determined quantitatively as described here. The quantity of cholesterol and coprostanol was determined colorimetrically in 2 ml. of glacial acetic acid and 4 ml. of Liebermann–Burchard reagent using suitable constants as previously described[4]. As a control the original corresponding aliquot was analysed colorimetrically by the direct addition of 2 ml. of glacial acetic acid and 4 ml. of Liebermann–Burchard reagent.

In the second series, mixtures of coprostanol and cholesterol were analysed in various proportions. In every case the mixtures totalled 800 μgm. of sterol. The sterols were analysed by the same method as described above and compared in each case with the absorbancy obtained for the appropriate control.

The results (Table 1) confirmed the complete recovery of cholesterol digitonide in all concentrations employed for either final volume. The photometric reading of coprostanol digitonide at the 300-μmgm. level is inaccurate and is omitted. The recovery of coprostanol of moderate to high levels in 3 ml. of solvent was found to vary from 97·1 to 70·6 per cent. When the final volume was doubled, an appreciable decrease in recovery was observed for comparable concentrations of coprostanol (65·0–59·6 per cent).

Table 1. RECOVERY OF CHOLESTEROL AND COPROSTANOL DIGITONIDES IN THE SPERRY–WEBB SOLVENT MIXTURE

Weight of sterol	Recovery of digitonide (percentage of total sterol)			
	Cholesterol		Coprostanol	
μgm	3 ml.*	6 ml.*	3 ml.*	6 ml.*
300	100	100	—	—
600	100	100	97·1	65·0
800	100	100	78·6	67·9
1,000	100	100	80·8	63·0
1,400	100	100	70·6	59·6

* The volumes indicated refer to the final volume of the solvent mixture.

Table 2. RECOVERY OF MIXED CHOLESTEROL AND COPROSTANOL DIGITONIDES IN THE SPERRY–WEBB SOLVENT MIXTURE

Composition of sterol mixture* (per cent)		Recovery of mixed digitonides (percentage of total sterol)
Coprostanol	Cholesterol	
0	100	100
25	75	89·4
50	50	88·6
75	25	78·9
100	0	78·6

* Total sterol, 800 μgm., 3 ml. of Sperry–Webb solvents.

Analysis of the mixtures of coprostanol and cholesterol revealed that increasing the proportion of coprostanol resulted in a decrease in the recovery of the mixed digitonide (89·4–78·6 per cent).

The quantity of sterol recovered from the Sperry–Webb washings was found to correspond in each case to that amount of sterol not precipitated by digitonin. Our present results suggests that incomplete precipitation of coprostanol digitonide in the solvents of the Sperry–Webb procedure or equivalent solvents may account for some of the observed quantities of 'non-3β-hydroxy fæcal sterols'[8]. Thus, it is our opinion that fæcal sterol analysis with the above limitations can be more successfully carried out by a suitable chromatographic procedure as previously described[4]. Recent developments in the separation of sterols by quantitative gas chromatography[9] have stimulated much interest in the applicability of this technique to quantitative sterol determinations.

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Action of Trypsin on Bence-Jones Protein Derivatives

It is well known that the proteolytic enzyme trypsin fails to hydrolyse native proteins, or hydrolyses them to only a very small extent. The denaturation of protein substrate is therefore a customary preliminary operation in all work involving the action of trypsin on proteins. We report here the effect of trypsin on some chemical derivatives of a pure de-ionized[1] Bence-Jones protein isolated in large amounts from the urine of a single patient.

The protein was oxidized with performic acid, as described by Harrington and Schellman[2], treated with sulphite by the Swan technique as modified by Bailey and Cole[3], reduced with mercaptoethanol in 8 *M* urea as suggested by White[4], or with sodium borohydride[5]. The completeness of reduction was checked by employing the *p*-chloromercuribenzoate spectrophotometric titration described by Sela *et al.*[6]. The reduction products were directly digested or previously carboxymethylated with iodoacetic acid or treated with iodoacetamide.

The trypsin employed was a salt-free twice crystallized commercial preparation (Sigma Lot *T* 30*B*–056).

All digestion experiments were performed in a *p*H-stat (model *TTT*–1*A* Radiometer, Copenhagen) employing 100 mgm. of the protein or its derivative in 4 ml. of de-ionized water brought to *p*H 8·00 with 0·2 *N* sodium hydroxide; 5 mgm. of trypsin in 0·5 ml. of 0·001 *N* hydrochloric acid added in five 0·1-ml. aliquots, each every 15 min. The *p*H was maintained at 8·00 with 0·2 *N* sodium hydroxide; the temperature was 30° C.

The molecular weight of this Bence-Jones protein was assumed to be 36,000 on the basis of physicochemical measurements and of quantitative N-terminal amino-acid determinations.

The results obtained are presented in Fig. 1. It is easy to see that the opening, either oxidative or reductive, of the disulphide bonds leads to the same sensitivity toward trypsin. Even the digestion-rates for the derivatives are similar, though the rate for the simply reduced protein (curve 4) is slower, owing probably to the combination of the free sulphydryl groups to form aggregates.

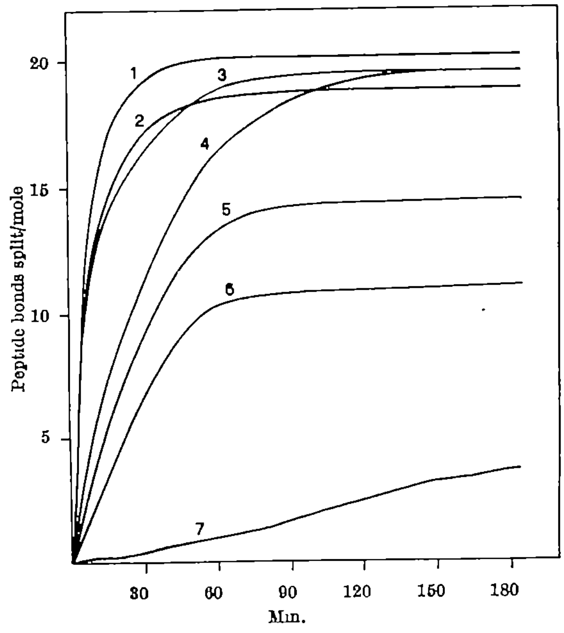

Fig. 1. Tryptic hydrolysis of Bence-Jones protein derivatives. Curve 1, sulphite-treated; curve 2, performic acid oxidized; curve 3, reduced and carboxymethylated; curve 4, reduced; curve 5, in urea 2 *M*; curve 6, in urea 1 *M*; curve 7, native

Bence-Jones protein in urea shows an increasing sensitivity to trypsin with increase of urea molarity (see curves 5 and 6).

It is concluded from the above results that anything which leads to a cleavage of disulphide bonds probably leads to a very similar configuration of the molecule, and that the urea-induced modifications, which respect the integrity of disulphide bridges, result in a lesser sensitivity to trypsin.

The use of tryptic digestion as an auxiliary tool for configurational studies on protein molecule is suggested.

Details of the above work and other results will be published fully elsewhere.

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Apparent Reversal of Xanthine Oxidase Action in *Chlorella vulgaris* starved of Nitrogen

In previous publications[1–3] a marked stimulation of the endogenous respiration of *Chlorella vulgaris* and other organisms by numerous purines and purine analogues was described. Several lines of evidence led to the suggestion that these results were best explained by a cyclical oxidation and reduction of the purines, coupled to oxygen on one hand and to the oxidation of fatty acids on the other. This assigns to xanthine oxidase a role in terminal oxidation. This communication presents evidence for an apparent reduction of oxidized purines in *Chlorella*, lending support to the existence of a purine oxidation-reduction cycle.

Chlorella vulgaris (ATCC 11468) was grown and harvested as previously described[1]. Washed cells were suspended in 0·067 *M* phosphate buffer, *p*H 6·0, and incubated with 10^{-4} *M* reduced purines (hypoxanthine or 8-azahypoxanthine) for 15 min before the oxidized purines labelled with carbon-14 were added (final concentration 10^{-4} *M*). After shaking the suspensions in the dark for another 30 min. solid trichloracetic acid was added to a final concentration of 5 per cent. The cell suspensions were centrifuged after 30 min. standing, and this extraction step was repeated twice. Authentic purines or azapurines were added as carriers. The extracts, freed of trichloracetic acid, were concentrated *in vacuo*.

Chlorella cells which had been exposed to hypoxanthine and then to uric acid-2-^{14}C were extracted. Fig. 1 shows the elution pattern of this extract from

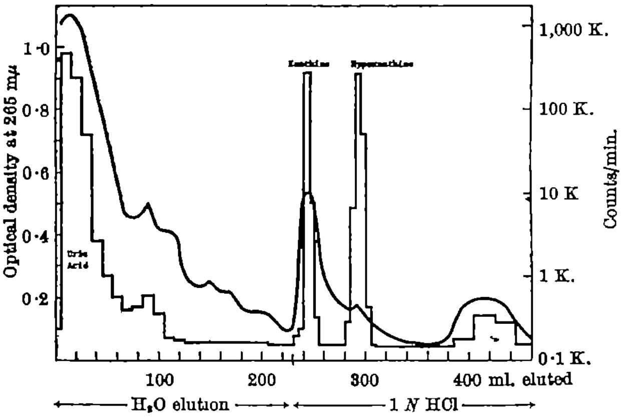

Fig. 1. Distribution of carbon-14 activity in the eluates from a 'Dowex-50(H$^+$)' column. An extract of cells which had been incubated with uric acid-2-^{14}C, together with uric acid, xanthine and hypoxanthine was applied. Boxed line, optical density; continuous line, radioactivity

a 'Dowex 50' column[4]. In addition to uric acid, considerable radioactivity was found in xanthine. Two other compounds also showed activity and were probably oxidation products of uric acid. Uric acid is oxidized rapidly in this system[1], and allantoin is eluted from this column directly following uric acid.

The peak corresponding to xanthine was freed of hydrochloric acid by evaporation to dryness *in vacuo*. The radioactivity in this peak co-chromatographed with authentic xanthine in the following solvent systems which were run in a descending manner: *n*-butanol/pyridine/water (70 : 30 : 10) run for 72 hr., *n*-butanol saturated with water (64 hr.), and *n*-butanol/methyl-ethyl-ketone/triethylamine/water (20 : 20 : 2 : 10) for 64 hr. The radioactivity could not be separated from the xanthine spot by high-voltage paper electrophoresis in 0·075 *M* phosphate buffer, *p*H 7·5.

Following exposure of *Chlorella* to 8-azaguanine-2-^{14}C, a very small amount of radioactivity was detected by paper chromatography of the cell extract in the 8-azahypoxanthine spot. These chromatograms were developed in an ascending manner in 2 *N* hydrochloric acid/*iso*propanol (35 : 65)[5]. However, the chromatograms were overloaded with azaguanine and azaxanthine and a second separation in the same solvent was necessary to obtain radiochemically pure azahypoxanthine. The doubly chromatographed azahypoxanthine was eluted and further rechromatographed in butanol saturated with water and also in butanol, pyridine, water. The material was also subjected to electrophoresis in 0·05 *M* sodium bicarbonate–sodium carbonate buffer, *p*H 9·85. With both methods the radioactivity was not resolved from the ultra-violet light-absorbing spot of 8-azahypoxanthine.

In addition to the previously demonstrated oxidation of the purines in *Chlorella*[6], these results show that a mechanism also exists whereby purines are reduced. These reduced products were present in small amounts and, therefore, the steady state for these reactions must be far towards oxidation. The level of radioactivity in hypoxanthine was probably too low for unequivocal detection.

The only previous report of a reversal of xanthine oxidase is that of Whitely and Ordal[7]. However, in this case the reaction was carried out in an atmosphere of hydrogen. My results are not sufficient to exclude the possibility that the apparent reversal of xanthine oxidase took place via a nucleotide cycle such as that described recently by Magasanik and Karibian[8]. Nevertheless, it is difficult to envisage the involvement of histidine biosynthesis in the purine effect, since this effect is dependent on nitrogen starvation of the cells and takes place in the absence of any added nitrogen sources. Furthermore, the instantaneous response of respiration to added purines[1] would not suggest that there is an initial conversion of the purines to the nucleoside or nucleotide stage. It has been found in results, which will be presented elsewhere, that the substrates for the purine-stimulated respiration are the storage lipids of the cells. It is tempting to speculate that the reduction of the purines is coupled in some way to a step in the oxidation of the lipids. The resulting cycle could serve as a mechanism for a terminal electron-carrying system.

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Action of Glutamic Acid and Glutathione Analogues on the *Hydra* Glutathione-Receptor

REDUCED glutathione (GSH) is known to activate surface receptors of *Hydra*, resulting in the co-ordinated feeding reflex[1]. Loomis's demonstration that asparthione (β-aspartylcysteinylglycine) did not activate the feeding reflex suggests that the γ-glutamyl moiety is essential for the action of GSH[1]. Furthermore, the demonstration by Cliffe and Waley that ophthalmic acid (γ-glutamyl-α-amino-*n*-butyrylglycine) activated the feeding reflex shows that the sulphydryl group is not necessary for the action of GSH on *Hydra*[2]. The present work was undertaken to characterize further the structural features of the tripeptide which are necessary for the activation of the GSH-receptor. In this series of experiments we demonstrate that either glutamic acid or glutamine act as competitive inhibitors of GSH. In addition, analogues of the GSH tripeptide were found either to activate or inhibit competitively the feeding reflex, depending on the size of the group substituted for the thiol.

The activity of the feeding reflex is expressed as previously described[3], and the experiments were carried out with the following modifications: (1) only *Hydra* starved for two days were used; (2) all experiments were carried out in a solution consisting

Table 1. ACTIVATION OR INHIBITION OF THE GSH-RECEPTOR

Compound	Duration of the feeding reflex (min.)		
	Compound alone	Compound + 10^{-6} M GSH	Compound + 10^{-5} M GSH
Control, no analogue added	—	28·3	35·2
Glutamic acid 10^{-5} M	0	0·8	22 0
Glutamine 10^{-4} M	0	0	23·1
Ophthalmic acid 10^{-5} M	31 6	—	—
S-methylglutathione 10^{-5} M	29 2	—	—
Asparthione 10^{-4} M	0	0	32·7
S-Acetylglutathione 10^{-4} M	0 9*	3 3	31·1
γ-L-Glutamyl-L-sulphi-alanylglycine 10^{-4} M	0	0	15 7
Oxidized glutathione 10^{-4} M	0	2 3	23 1

* A small amount may be hydrolysed at these conditions.

of 10^{-3} M calcium chloride, 10^{-4} M sodium chloride and 10^{-3} M histidine buffer, pH 6·2.

The results (Table 1) demonstrate that 10^{-5} M glutamic acid inhibited the feeding reflex which was activated by 10^{-6} M GSH. Increasing the GSH concentration to 10^{-5} M reversed this inhibition; thus, the inhibition is thought to be competitive. Glutamine, at 10^{-4} M, also was shown to inhibit competitively. Not inhibitory were cysteine, glycine, α-ketoglutaric acid, glutaric acid, pyroglutamic acid, aspartic acid, asparagine, and glycylglycine.

Also shown in Table 1 are the actions on *Hydra* of different GSH analogues. Only two, ophthalmic acid and S-methylglutathione, activated the feeding reflex in the absence of GSH. Analogues having large substituents at the thiol position, as in the case of S-acetylglutathione, oxidized glutathione, or γ-L-glutamyl-L-sulphi-alanylglycine, did not activate the feeding reflex. In contrast, 10^{-4} M concentrations of the analogues were potent inhibitors of GSH at 10^{-6} M. These inhibitions were partially or completely overcome by increasing the GSH concentration to 10^{-5} M.

It is of interest to compare the activity of GSH analogues on two different systems requiring GSH: the enzyme glyoxalase, and the GSH-receptor of *Hydra*. Glyoxalase requires a tripeptide coenzyme which must be both similar to GSH and contain the free sulphydryl group; for example, asparthione is nearly as effective as GSH[4]. All analogues having the thiol group modified, but retaining the tripeptide structure, are competitive inhibitors (see refs. 5 and 6).

The GSH-receptor of *Hydra*, on the other hand, does not require the thiol group for activity; in this category ophthalmic acid and γ-glutamylalanylglycine[2] and S-methylglutathione (Table 1) are activators. Yet related analogues (ref. 2 and Table 1), having unusually large substituents for the thiol group, do not activate but apparently occupy the receptor site and behave as competitive inhibitors. In contrast to glyoxalase, the GSH-receptor is very sensitive to changes in the glutamyl moiety of GSH, since asparthione does not activate the GSH-receptor but acts as a competitive inhibitor. Furthermore, glutamic acid, while competing with GSH for the GSH-receptor, has no effect on glyoxalase[5].

The part played by the glycyl moiety remains obscure. Glycine alone does not seem to be tightly bound by the receptor as indicated by its failure to act as a competitive inhibitor. None the less, Loomis's demonstration that γ-glutamylcysteine does not activate the receptor[1] indicates that the glycine part of GSH is necessary.

An analysis of the data (Loomis[1], Cliffe and Waley[2], and Table 1) indicates that the GSH-receptor has a most unusual specificity when compared to other proteins reacting with GSH. It is concluded that in order to activate the GSH-receptor, the structure of the tripeptide 'backbone' must be identical to that of GSH, while the thiol group, branching off the 'backbone', can be altered within certain limits.

We wish to thank Dr. S. G. Waley and Dr. V. du Vigneaud for their generous gifts of ophthalmic acid and γ-L-glutamyl-L-sulphi-alanylglycine, and asparthione respectively. Dr. J. F. Woessner's valuable criticisms of this manuscript are appreciated.

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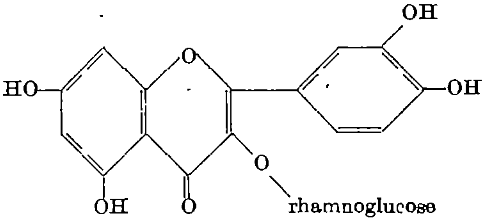
Isolation of Chromatographically Pure Rutin from Flowers of Elder

RUTIN (3-rhamnoglucoside of 5,7,3′,4′-tetrahydroxyflavanol) (I), one of the most widespread flavonoids, has been found in numerous plant materials[1,2]. On the commercial scale for pharmaceutical purposes it is usually manufactured by isolation from stalk and leaves of buck-wheat (*Fagopyrum sagittatum*)[3,4] and from the Japanese pagoda tree (*Sophora japonica*)[5]. A chemically pure preparation is then obtained by repeated recrystallization of the crude product.



(I)

Within the framework of a study of isolation of flavonoid substances from natural material it has been found that chromatographically pure rutin can be easily isolated from flowers of the elder (*Sambucus nigra*). For this purpose chromatographical partition of the extract on powdered polyamide ('Silon') material has been successfully used.

Chromatography on powdered 'Silon' has been used partly for the separation of flavonoid substances from some natural materials[6–8] and partly in their chemical analysis[9].

Dried elder flowers are extracted in a Soxhlet apparatus with 75 per cent methanol so long as the extract is green–yellow coloured. The extract is then vacuum concentrated and afterwards diluted with water in the ratio of 1 : 1. The resulting sediment of chlorophyll is filtered away, and the filtrate concentrated once more. The extract thus prepared is chromatographically purified on powdered polyamide (granulation 0·1–0·4 mm.). The size of the chromatographical column is dependent on the quantity of the extract. The polyamide powder is first allowed to swell in distilled water for about 1 hr., then the suspension is transferred to the chromatographic column and settled by gentle tapping. The concentrated extract is then passed through the column. Elution is carried out first with water and afterwards with 30 per cent methanol. The water part contains mostly the sugar ballast components ; the methanolic fraction the flavonoid substances. The course of elution is watched by means of an ultra-violet lamp, and the fraction showing a brown-green fluorescence is collected. The methanolic eluate is concentrated to a small volume and the resulting sediment of rutin filtered away and dried. By this method it is possible to obtain pure rutin from elder flowers. It has been found that the flowers contain as much as 3 per cent of rutin in the dry residue. The method described makes it possible to recover as much as 90 per cent of rutin which is chromatographically pure.

The melting point of the obtained rutin and the R_F in the solvent system *n*-butanol/acetic acid/water in the ratio 4 : 1 : 2·2 proved to be identical with those of a pure preparation. Acid hydrolysis yields quercetin; from the sugars, glucose and rhamnose in the ratio 1 : 1 have been found. Chemical analysis of the product was also in agreement with pure rutin.

This method of isolating chromatographically pure rutin from elder flowers by partition on powdered polyamide is rapid. simple and easy to carry out. The method is preferable also from the economical point of view, since a chromatographically pure preparation is obtained without any recrystallization.

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Estimation of Volatile Anæsthetics in Tissues by Gas Chromatography

IN work on the uptake and elimination of volatile anæsthetics by the body, a convenient method is needed to determine their concentrations in blood, brain, liver, muscle, fat, etc. The elimination of these anæsthetics from the blood follows an exponential law. To determine the elimination curve accurately, a large number of samples has to be taken. It is desirable that the total volume of blood removed be as small as possible, and the method has, therefore, to be as sensitive as possible. This applies particularly to the last part of the curve, when the concentrations of the anæsthetic are low, for example, 10–1 p.p.m.

Conventional methods of estimating di-ethyl ether[1], chloroform and trichloroethylene[2] and halothane[3] are unsatisfactory because they require relatively large samples, lack specificity, and are time-consuming. With these methods concentrations less than 10 p.p.m. are difficult to estimate. Mass or infra-red spectrometry has a sensitivity which is less, that is, about 100 p.p.m. for detection and about 1,000 p.p.m. for estimation.

An elegant solution to the problem is the use of gas chromatography[4]. A method of utilizing the blood sample directly is being developed. At present, however, the anæsthetic is extracted from blood (or other tissue) into *n*-heptane. Using gas chromatography, it is possible to estimate halothane in *n*-heptane at concentrations less than 10 p.p.m.

The separation of *n*-heptane and the anæsthetic is achieved in a column 12 in. long. The column is made of copper refrigeration tubing, ¼ in. internal diameter, packed with 80–100 B.S. mesh 'Celite', which is impregnated with 10 per cent by weight of silicone fluid M.S. 550. The column is maintained at a steady temperature in an air oven. Hydrogen is used as the carrier gas at a flow-rate of about 45 ml./min. Extracts containing more than one substance may require a longer column, perhaps 30 in. long. The temperature of the column should be 25–30° C. for di-ethyl ether and halothane, and for substances with higher boiling points, for example, chloroform, trichloroethylene and methoxyflurane, it should be 100–120° C.

A flame ionization detector modified from that of Harley *et al.*[5] has proved suitable. Gauze electrodes, made of stainless steel, are used on either side of the flame, but not in contact with it. This type of detector appears more sensitive to halogenated compounds than the usual flame detector[6]. A potential difference of 120 V. is placed across the electrodes, and the ionization current is passed through a 1,000 megohm resistor. The resulting potential is measured with a simple balanced cathode follower unit or a vibrating reed electrometer, feeding a potentiometric recorder. Each sample is introduced into the column with a

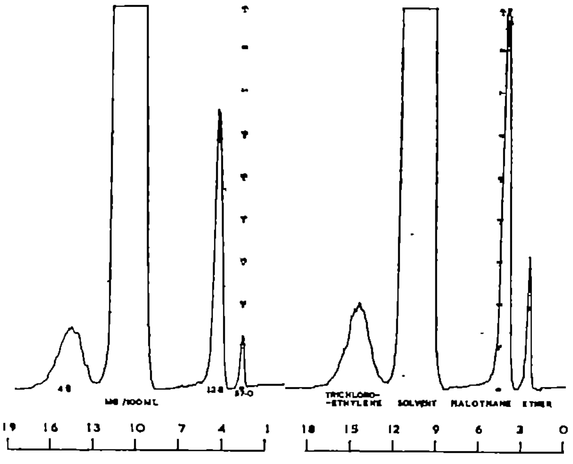

FIG. 1. Chromatogram showing di-ethyl ether, halothane and trichloroethylene after their extraction from blood with *n*-heptane. 'Celite'–silicone column at 30° C. ; carrier gas at 45 ml. per min. ; 12 in. chart at ⅓ in. per min. The approximate concentrations are shown beneath the peaks. On the second run (left) the gain was reduced so that the halothane peak could be estimated

capillary pipette[7], delivering 0·15–0·50 µl. The technique for introducing the sample is carefully standardized.

Using this method, halothane can be measured at a concentration of about 10 p.p.m. and a calibration curve had a coefficient of variation of 0·68 per cent. When the peaks on the chromatogram are narrow, their heights rather than their areas may be used for making standard curves. Fig. 1 illustrates the ability of the method to separate and detect different anæsthetics which had been extracted from one sample of blood.

We wish to acknowledge the encouragement of Prof. R. F. Woolmer in this work, and the financial support of the Sir Halley Stewart Trust.

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3-Aminomethylindole and 3-Methylaminomethylindole: New Constituents of Barley

THIS communication reports the identification of two new alkaloids, related structurally and perhaps biosynthetically to gramine. Shoots of 4-day-old barley seedlings were extracted with trichloracetic acid. The centrifuged solution was extracted with ethyl ether, made alkaline and re-extracted. The ether extract of the alkaline solution was dried and concentrated. Aliquots were chromatographed using paper impregnated with a mixture of 0·2 *M* dipotassium hydrogen phosphate and 0·03 volume of 0·1 *M* citric acid[1] and developed with *iso*propanol equilibrated with 0·29 volume of the same buffer. Sprayed with Millon's reagent[1], the chromatograms showed a strong gramine spot, and two spots called here compounds *A* and *B* and identified by the evidence below as 3-aminomethylindole and 3-methylaminomethylindole respectively. In addition, there was an unidentified spot.

The rate of movement of compound *A* relative to that of gramine (R_{Gramine}) is approximately 0·66. It reacts with Millon's reagent to form a yellow-brown spot which gradually acquires a reddish hue, gives a yellow spot with ninhydrin, but no colour with either the benzoquinone or the nitroprusside reagents used to detect secondary amines[2]. Eluted from a paper chromatogram and dissolved in dilute hydrochloric acid, this material displayed a distinctive ultra-violet absorption spectrum in all respects similar to the spectra of authentic 3-aminomethylindole and gramine.

In the presence of compound *A* and of methyl-^{14}C-(–)-S-adenosyl-L-methionine, an enzyme preparation from barley shoots forms a new radioactive compound (R_{Gramine} = 0·76). The reaction is dependent on compound *A*. This radioactive compound on treatment with excess methyl iodide in ethanol gives rise to further radioactive spots which were identified chromatographically as gramine and tetramethylammonium salt, and to material(s) which move with an R_{Gramine} of approximately 1·19. These results are entirely compatible with the postulate that compound *A* (3-aminomethylindole) is converted (enzymatically) to 3-methylaminomethylindole. The latter, on treatment with methyl iodide, is changed to gramine, the methyl moiety of which in the presence of methyl iodide is known to be recovered both in tetramethyl-ammonium ion and in other compounds[3].

Authentic 3-aminomethylindole, prepared essentially by Putochin's method[4], displays properties similar to those of compound *A* in all these particulars.

Compound *B* moves with an R_{Gramine} of 0·76, reacts with Millon's reagent to give a final colour intermediate between those of compound *A* and gramine, gives no colour with ninhydrin, but reacts as a secondary amine with the sprays mentioned previously. After elution from a preparative chromatogram, compound *B* has an ultra-violet spectrum in dilute hydrochloric acid similar to that of gramine. Naturally occurring compound *B* is indistinguishable from the compound formed by the enzymatic or (in poor yield) chemical methylation of 3-aminomethylindole. It was further shown that an enzyme obtained from barley shoots forms radioactive gramine when incubated with methyl-^{14}C-(–)-S-adenosyl-L-methionine, this reaction being dependent on the addition of compound *B*.

Taken together, these findings establish the identity of compound *A* with 3-aminomethylindole and of compound *B* with 3-methylaminomethylindole.

Work on the metabolism of these substances is continuing, and will be reported on fully. At present it may be suggested that gramine is formed physiologically by the enzymatic transfer of methyl groups from (–)-S-adenosyl-L-methionine to 3-aminomethylindole to form successively 3-methylaminomethylindole and gramine.

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PHYSIOLOGY

Local Treatment of Experimental Burns with a Monoamine Oxidase Inhibitor

PREVIOUS work has shown that prior systemic administration of inhibitors of the enzyme monoamine oxidase led to striking diminution of the increased capillary permeability that followed thermal and chemical injury in rats[1]. Similar anti-inflammatory properties have been reported independently[2]. This effect may be due to the preservation of an adrenaline-like substance the destruction of which by monoamine oxidase might permit the full development of the inflammatory reaction. The present investigation was undertaken to determine whether monamine oxidase inhibitors would reduce inflammatory œdema when applied locally after thermal injury.

Standard thermal injury, in this case 60° for 27 sec., was produced in anæsthetized rats by methods previously published[3]. The monoamine oxidase inhibitor employed was (2-4′-hydroxyphenoxyethyl) hydrazinium chloride (compound 1273, Smith and Nephew Research, Ltd.). The substance was made up as a 5 per cent ointment in a 'Carbowax' base (polyethylene glycol 400, 60 per cent; polyethylene glycol 4,000, 35 per cent). The ointment was applied immediately after completion of the period of thermal injury. It was rubbed in gently but liberally and continuously for 15–20 min., the animals then being returned to their cage. Three hours after thermal injury the rats were killed and the burnt area excised for the assay of inflammatory œdema as described previously[3]. Control rats consisted of untreated animals and of animals treated identically, but with the ointment base alone.

The results were expressed as gm. water/cm.^{2} skin, and are set out in Table 1. In order that the figures given should truly represent inflammatory œdema, the normal water content of skin (0·03 gm./cm.^{2}) was deducted from each set of figures before setting out the table.

Table 1. Effect of Monoamine Oxidase Inhibitor Ointment on Œdema after Standard Thermal Injury

Treatment	No. of animals	Mean value water content of burnt area (gm./cm.^{2})	Range of values (gm./cm.^{2})	P
None	12	0·21	0·13–0·35	—
Compound 1273 ointment	10	0·09	0·09–0·10	< 0·001
Ointment base only	6	0·22	0·13–0·35	—

It can be seen that thermal injury of this intensity led to a very considerable œdema of skin and subcutaneous tissues. It is equally apparent that application of the ointment containing compound 1273 led to a striking diminution of this increased capillary permeability, whereas similar use of the ointment base alone failed to do so.

It is reasonable to suppose that monoamine oxidase inhibitors have a similar effect when administered locally as an ointment or systemically, that is, by preventing the inactivation of an endogenous adrenaline-like substance the action of which is to diminish capillary permeability to protein[4].

This hypothesis is supported by the inhibition of inflammatory œdema that results from the subcutaneous injection of adrenaline (0·5 mgm./kgm.) up to 30 min. after standard thermal injury (Spector and Willoughby, unpublished observation). On the other hand, intravenous injection of monoamine oxidase inhibitors after thermal injury failed to diminish increased capillary permeability. If ointment and injection operate on similar endogenous mechanisms the discrepancy between their effectiveness after thermal injury must be presumed to be due to better access to the affected tissues by the locally applied substance. Again, if ointment and prior injection work in similar fashion, the present experiments provide further evidence to indicate that, as suggested previously[4], release and destruction of the adrenaline-like substance is a local phenomenon at the site of injury.

We thank Messrs. Smith and Nephew Research, Ltd., for their co-operation, and Sir Roy Cameron for his interest in the investigation.

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Augmentation of the Pressor Response to Guanethedine by Inhibition of Catechol O-Methyltransferase

Intravenous administration of guanethedine has been reported to cause a pressor response[1,2], contraction of the nictitating membrane of the cat and pilo-erection[2]; the pressor response was not blocked by ganglionic blocking agents[2]. These phenomena lead to the possible conclusion that guanethedine causes a release of catecholamines or has some direct adrenergic effects.

The catecholamines are biochemically inactivated by the enzyme catechol O-methyltransferase[3] and this enzyme is inhibited under both *in vivo* and *in vitro* conditions by various polyphenols[4] which compete with the catecholamines for the enzyme[5]. The results of Wylie *et al.*[6] showed that the effects of both endogenous and exogenous catecholamines could be prolonged by administration of the catechol O-methyltransferase inhibitor pyrogallol; this type of agent, therefore, provides a specific tool for the pharmacological determination of the release of catecholamines.

In the following experiments spinal cats were used, and the blood pressure was determined by cannulation of the carotid artery. In all experiments responses to *l*-adrenaline, *l*-noradrenaline and guanethedine were obtained before and after pyrogallol. The doses of all substances are given as base. The areas of the pressor responses were measured in cm.^{2} by means of a planimeter.

In the first series of experiments on 6 cats the average response to a total of 15 injections of 200 μgm./kgm. of guanethedine was 1·68 ± 0·19 (*S.E.*) cm.^{2}. Two min. after administration of 4 mgm./kgm. of pyrogallol to each of the 6 cats, the average response to the standard dose of guanethedine was 5·09 ± 0·26 cm.^{2}, and 15 min. later was 3·87 ± 0·19 cm.^{2}. The average response to a third injection 32 min. after administration of the pyrogallol was 2·12 ± 0·21 cm.^{2}. Thus the pyrogallol markedly augmented the pressor response to guanethedine, and as shown in previous work[6] this action of pyrogallol lasted for about 30 min. In none of these experiments did this dose of guanethedine produce a preliminary hypotension as reported by others[1,2].

Many substances have been reported to release catecholamines from the adrenal glands. In 3 cats the effects of guanethedine alone and immediately following pyrogallol were measured before and after acute bilateral adrenalectomy. In all cats removal of the glands reduced the blood pressure by about 20 mm. mercury; but the responses to guanethedine

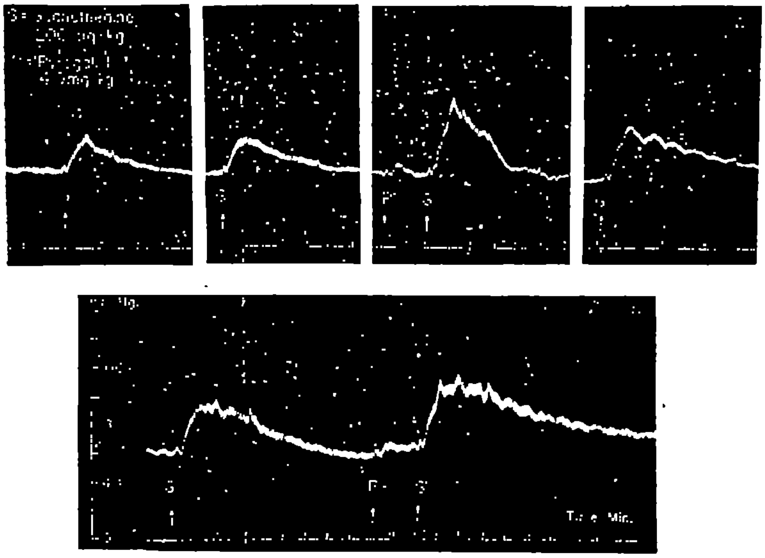

Fig 1. Pressor responses to guanethedine (*G*) and pyrogallol (*P*) plus guanethedine, before and after bilateral adrenalectomy between tracings *A* and *B*

were similar to those before adrenalectomy and could still be augmented by pyrogallol. The tracing obtained during one of these experiments is shown in Fig. 1.

It would therefore appear from the results given here that guanethedine causes a pressor response by the indirect mechanism of releasing catecholamines as this response is augmented by prior administration of pyrogallol, a known catechol O-methyltransferase inhibitor. As the pressor response is not diminished by ganglionic blocking agents[4] and is not much affected by bilateral adrenalectomy, it becomes more probable that guanethedine liberates catecholamines from the sympathetic nerve endings. Such a mechanism of action would explain the sympathetic atony reported by Maxwell *et al.*[2] to follow several hours after medication.

I am grateful to Mrs. M. Hart for assistance.

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Rate of Evaporation from Different Regions of the Skin of Indian Zebu Cattle

Indian zebu cattle differ from the cattle of the temperate regions in having a marked hump and voluminous dewlap and navel flap. These structures are specially well developed in areas with very oppressive summer heat, and it has been suggested that these special structures may be related to the animal's greater thermoregulatory power.

The distribution of sweat glands has been studied in the dorsal and ventral skin and in hump, dewlap and navel flap. The secreting surface area of a single sweat gland is not significantly higher in the hump, but the effective secreting surface per sq. cm. of skin surface is much higher owing to a very high concentration of sweat glands[1].

An attempt has been made to study the physiological function or the activity of the sweat glands by determining the evaporation of sweat from different cutaneous surfaces by Berman's method[2]. Cobalt chloride anhydride paper disks (28·3 sq. mm.) were applied on different areas of the body and the time for the complete change of colour noted at an ambient temperature of 38° C. and 74 per cent relative humidity. The average time taken for the dorsal trunk to decolorize the disks was 3 min. 17 sec., and taking this as the standard 100 per cent evaporation, the relative evaporation-rate from other areas was 149 per cent on the hump and 124 per cent on the neck; but the rate on the abdomen was only 72 per cent and on the dewlap and sheath was only 58 per cent. Thus the hump has developed special thermoregulatory significance in the tropical zebu cattle. These findings also confirm the work on the Brahman cattle[3].

We are indebted to I.C.A.R. for grants-in-aid to defray the expenses of the investigation and to Lieut.-Colonel J. M. Lahiri, director of veterinary services and animal husbandry, West Bengal, and to Principal K. C. Kukherjee for their interest and advice.

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Inability of Refined Corn Oil to influence Spontaneous Arteriosclerosis of Old Hens

According to a large number of reports, vegetable oils (such as corn, cotton-seed, rape-seed, safflower oil, etc.) are able to decrease serum cholesterol both in man and experimental animals. Some evidence exists that this effect is, at least partly, related to the presence of highly unsaturated fatty acids such as linoleic acid. It is not clear, however, whether such vegetable oils also have beneficial effects on arteriosclerotic lesions. After cholesterol-feeding, conflicting results have been reported with rabbits as well as with cockerels. Experimental arteriosclerosis due to cholesterol-feeding may, however, be different from arteriosclerosis in man. Probably the spontaneous arteriosclerotic lesions in old birds, for example hens[1], are more like human arteriosclerosis.

Table 1. INFLUENCE OF CORN OIL FEEDING ON SPONTANEOUS ARTHRIOSCLEROSIS IN THE AORTA OF OLD HENS (AVERAGE ± MEAN ERROR)

Animals receiving*	Macroscopic lesions				Total lipids‡ percentage of:		Total cholesterol§ percentage of:		
	Arcus aortæ†	Aorta thor.†	Aorta abd.†	Percentage of total aorta	Fresh weight	Dry weight	Fresh weight	Dry weight	Total lipids
Lard + coconut fat (controls)	1	3±0·6	3±1·7	18±3	3·59±0·17	14·2±1·6	0·38±0·01	1·55±0·13	12·25±6·55
Corn oil	1	2±0·4	2±0·6	15±4	3·81±0·21	14·3±1·5	0·39±0·06	1·62±0·09	11·83±1·96

* See Fig. 1.
† Visual approximation on a 0–6 scale.
‡ The aorta was carefully freed from fat and connective tissue and extracted (ref. 4). The total lipids were purified (ref. 6) and weighed.
§ Liebermann–Burchard reaction with digitonin-precipitable substances of unsaponifiable material.

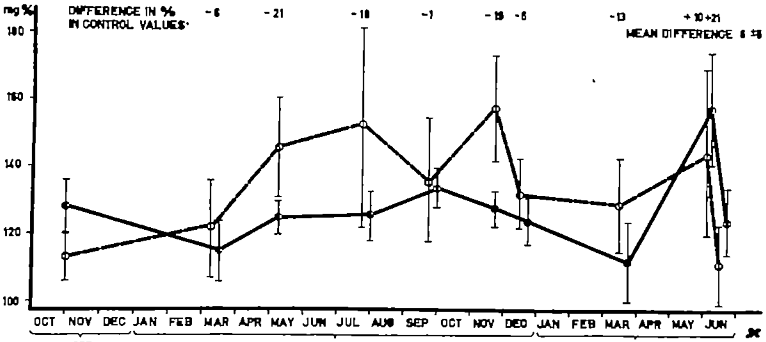

Fig. 1. Influence of dietary corn oil on serum cholesterol (ref. 5) in old arteriosclerotic hens. Points represent average values, vertical lines indicate error. – – –, Controls; ——, corn oil group

We have therefore investigated the effect of dietary corn oil on spontaneous arteriosclerosis of old hens.

Partridge-coloured Italian hens of about two and a half years were fed for 20 months a mash containing 5 per cent corn oil or 5 per cent highly saturated fats respectively. At the beginning of the experiment, randomly selected animals showed fewer aortic lesions than controls at the end of the experiment. Thus the influence of corn oil on existing and progressing arteriosclerosis was investigated.

Diet of controls: 3·0 per cent lard and 2·0 per cent coconut fat (= about 11 per cent of total calories); 6·3 per cent de-skimmed milk powder; 9·3 per cent meat powder; 8·9 per cent yeast; 3·5 per cent hay powder; 24·7 per cent rolled oats; 20·5 per cent wheat grains; 20·8 per cent wheat, rough-ground; 0·9 per cent sodium chloride; 0·1 per cent ferric citrate; 0·01 per cent manganese sulphate.

Number of animals at the end of experiment: 8 (4 animals lost in the course of the experiment because of 'diphtheria', peritonitis, tumour, etc., are not considered). Average body-weight: 1·79 kgm. at the beginning, 2·04 kgm. at the end ($\Delta = +0·29$ kgm.).

Diet of corn oil animals: 5·0 per cent corn oil (refined, edible, winterized quality, E. F. Drew and Co., Inc., Bonnton, New Jersey; content of linoleic acid 54·2 per cent, peroxide number 3·85 (analysis by Dr. J. Hofstetter, F. Hoffmann–La Roche and Co., Ltd., Basle) instead of lard and coconut fat.

Number of animals at the end of experiment: 10 (3 lost by similar diseases as controls were not considered). Average body-weight: 1·67 kgm. at the beginning, 2·03 kgm. at the end ($\Delta = +0·39$ kgm.).

Fig. 1 shows that dietary corn oil failed to diminish total serum cholesterol. Although in the corn oil group a tendency to decreased levels was obvious, the differences from controls were not significant ($P > 0·05$). This finding is in agreement with observations in mature cockerels[2] and growing chicken[3], the serum cholesterol of which was not decreased by oils rich in highly unsaturated fatty acids.

In fowls, the degree of cholesteræmia does not necessarily parallel that of spontaneous arteriosclerosis[4]. Therefore the condition of the arterial tissue itself is of more interest. In the present experiments, however, visible lesions as well as total lipids and cholesterol of the aorta were also not influenced significantly by the corn oil diet ($P > 0·05$) (Table 1).

Thus, in a long feeding period, 'physiological' amounts of refined edible oil (containing 54 per cent of linoleic acid) failed to diminish spontaneous arteriosclerosis of the aorta in old hens.

The conflicting results with cholesterol-fed rabbits and cockerels, as well as the present negative findings in old hens, do not support the assumption that linoleic acid of vegetable oils has beneficial effects on the development of arteriosclerosis. It remains, however, to be shown whether a higher percentage of corn oil in the diet is needed, or whether hens do not respond like mammals. Before these possibilities are excluded, definite conclusions regarding human arteriosclerosis cannot be drawn.

Addendum. Since submission of this manuscript, it has been reported that even a double amount of corn oil in the diet (10 per cent) fed to Leghorn pullets for the following three years did not affect the development of spontaneous lesions of the abdominal aorta in comparison with controls receiving animal tallow[7].

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Effects of Pulped Soybean Silage on the Milk-yield of Cows

As a result of co-operative investigations carried out with the Research Institute for Animal Physiology, a report has already been published[1] on the summer feeding of green soybeans, in which it was shown that the relative protein-deficiency in green maize silage having a high dry-matter yield may be compensated by the flowering, pod-forming soybean. In the present communication we propose to describe our experimental results on the feeding of silage

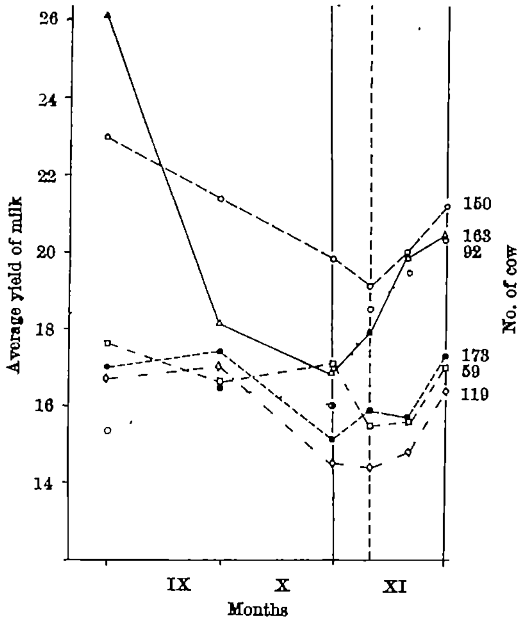

Fig. 1. Milk-production curve during feeding with soybean silage

made from soybeans pulped in the green condition. The analytical results showed that the digestible protein content and starch value of 1 kgm. of wheat bran are equal to the digestible protein content and starch value of 5 kgm. pulped soybean silage. We endeavoured, therefore, to demonstrate in our feeding experiments with pulped soybeans whether from the point of view of food conversion wheat bran can be substituted by soybean-silage.

The wheat bran fed in the preparative and first phase of the experiment was gradually substituted by soybean silage, as shown in Table 1.

Table 1

Experimental phase	Days	Quantity of food fed daily (kgm.)					
		Alfalfa	Best	Chaff	Bran	Maize meal	Soybean silage
Preparative	5	7	15	4	3	2	—
Phase I	5	8	15	3	3	2	—
,, II	5	8	15	3	2	2	5
,, III	5	8	15	3	1	2	10
,, IV	10	8	15	3	—	2	15
Concluding	5	7	15	4	3	2	—

Table 2

Experimental phase	Daily average milk production	Increase in yield in relation to the preparative phase	Relative growth of butterfat (per cent)
Preparative phase	16·2	—	—
Phase I	16·9	0·7	0·1
Phase II	17 3	1·1	0·2
,, III	17·5	1 3	0·2
,, IV	18·8	2 6	0·3

Table 2 shows that there was a considerable increase in the daily average milk production compared with the average shown during the preparative period.

The increased average yield of milk and of its percentage of butterfat contents indicate that soybean-silage is not only a substitute for the wheat bran, but also, due to its stimulating action on milk production, is from the biological and physiological aspects a far more valuable food than wheat bran, although both have the same nutritional value. Indeed, the food conversion value of soybean silage, taking wheat bran as 100 per cent, is 116 per cent. The effect on the lactation curve of feeding soybean silage is illustrated in Fig. 1. We believe that the break in the course of the lactation curve, when it turns upwards, should be interpreted as a hormone effect. Among the large number of physiologically active substances occurring in the soybean only a few may be mentioned the role of which is presumably not negligible: hæmatin, α-lipoic acid, vitamin E (tocopherol), vitamin K (α-phylloquinone), vitamin B_6 (adermin), pantothenic acid, stigmasterin, sitosterin, campesterin, ergosterin, sapogenol(s), etc. (Karrer[2]). According to Magee and Matrone[3], the œstrogen-like effect of green soybeans stimulates the development of the uterus in mice, and we believe a part is played by the high sterin content of the soybean. The increase of the butterfat content during feeding with soybean silage is equally indicative of the functional activity of acetyl-coenzyme *A* and may perhaps be connected with the relatively high acetyl-coenzyme *A* content of the soybean. It is noteworthy that the relatively high palmitic acid content of soybean oil, and easier linkage of the palmitic acid into the fatty acid cycle, may be, according to Green[4], related to the increased percentage of butterfat during feeding with soybean silage.

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Antiaccelerator Action of Methoxamine

In 1949 it was demonstrated by Otto Krayer[1] that the heart-rate-increasing action of adrenaline could be selectively antagonized by veratramine through a direct inhibitory action on the pacemaker of the heart. Since then it has become established that secondary amine bases of steroid nature among veratrum alkaloids all share this property in common and the term 'anti-accelerator' was given to this property[2]. More recently this property has been recognized as occurring in several types of compounds other than veratrum alkaloids[3,4].

In the course of the systematic investigations of the cardiac action of β-hydroxy-β-(2,5-dimethoxyphenyl)-*iso*propylamine (methoxamine), a synthetic sympathomimetic amine, it was found that in certain concentrations this compound also was capable of antagonizing the cardioaccelerator action of adrenaline, while it produced itself almost no change in the heart-rate. As such a property in a sympathomimetic amine is very interesting because it may provide an important clue to the better understanding of the basic mechanism of the action of the sympathomimetic amines upon the heart, we wish to report the findings briefly. The full account will be published elsewhere in the near future.

The effect can best be demonstrated in the isolated atropinized mammalian heart. We used the heart—

lung preparation of the dog and performed the following two types of experiments.

(1) *Modification of the chronotropic effect of single doses of adrenaline.* When 2–5 µgm. of adrenaline was injected in the heart–lung preparation of the dog, the heart-rate increased considerably to reach a peak in 20 sec. or so and then gradually decreased to the initial level. A second injection proved to be as effective as the first one in its cardioaccelerator action; the same degree of increase in the heart rate occurred and the time course was practically unchanged. However, after administration of 10–20 mgm. of methoxamine, the heart became less sensitive towards the cardioaccelerator action of adrenaline. In a typical experiment administration of 2 µgm. of adrenaline increased the heart-rate from 103 to 157 (increase of 54 per cent). 8 min. after 20 mgm. of methoxamine, the same dose of adrenaline increased the heart-rate from 113 to 121 (increase of only 7 per cent). Later, the effect continued undiminished, and 30 min. after methoxamine the increase in the heart-rate was 118 to 124 (increase of 5 per cent).

(2) *Effect of methoxamine on the cardioaccelerator action of continuous adrenaline infusion.* When adrenaline was administered continuously at a rate of 2–4 µgm. per min. in the heart–lung preparation of the dog, the heart-rate increased gradually to a steady level, its height depending on the rate of administration. Once a steady level was reached, the heart-rate decreased little over a period of as long as 3 hr. When methoxamine was given, in doses of 1·0–100 mgm., to a heart–lung preparation with an augmented heart-rate due to continuous infusion of adrenaline, a decrease in the heart-rate occurred; 1·0 mgm. showed a noticeable, 5 mgm. a marked, decrease; 20 mgm. reduced the acceleration by about 50 per cent and 100 mgm. caused a decrease almost to the initial value (Fig. 1). The steepest fall in the heart-rate occurred during the first 2 or 3 min.; but some further deceleration usually proceeded over a period of subsequent 5–10 min., until a new level was reached. There is a quantitative relationship between the dose of methoxamine and the degree of deceleration. The dose of methoxamine causing 50 per cent inhibition of acceleration was 20 mgm. or thereabouts, a value which makes the anti-accelerator potency of methoxamine approximately one-hundredth of that of veratramine. The nature of this antagonism seems to be competitive, for, even at a time when a certain infusion-rate of adrenaline could no longer increase the heart-rate as the result of inhibitory action of methoxamine, a sufficiently high concentration still was able to give full scope to its characteristic positive chronotropic effect. Thus ten times as much as the initial infusion-rate of adrenaline readily overcame the reduced sensitivity of the heart and led to a very marked increase in the heart-rate (Fig. 1).

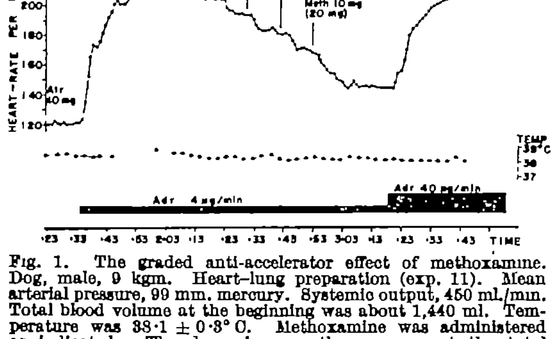

Fig. 1. The graded anti-accelerator effect of methoxamine. Dog, male, 9 kgm. Heart–lung preparation (exp. 11). Mean arterial pressure, 99 mm. mercury. Systemic output, 450 ml./min. Total blood volume at the beginning was about 1,440 ml. Temperature was 38·1 ± 0·3° C. Methoxamine was administered as indicated. The doses in parentheses represent the total amount of methoxamine administered up to the time of the respective signals

Since these actions were observed in the isolated atropinized heart, there is no doubt that they arose from the direct action of this compound on the heart muscle. A recent report from our laboratory has shown that methoxamine reduced the oxygen consumption of the isolated perfused dog heart[5]. Dealing with the protecting action of methoxamine against the toxic effect of X-ray irradiation, Smith *et al.* in their discussion[6] have suggested the reduction of metabolic-rate as the possible cause of the protection. If methoxamine has an inhibitory action on the tissue metabolism as these results imply, such an inhibition of metabolism could be the cause of the above-mentioned antagonism of methoxamine to adrenaline.

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Uptake of Small Resin Particles by the Alimentary Canal of the Calf

The demonstration by Payne, Sansom, Garner, Thomson and Miles[1] that small resin particles can penetrate the wall of the alimentary tract of the calf is clear, but their claim that similar processes may be of nutritional significance is open to doubt.

The highest concentrations of particles detected were in lymph nodes and were all less than $2 \cdot 5 \times 10^3$ c.p.m./10 gm. Even if so high a concentration had been found throughout all the tissues of the animal, the recovery of the radioactivity from an animal weighing 50 kgm. would have been only just a little more than a two-thousandth of the dose administered. As the lymphatic tissue forms a trivial fraction of the body, as muscle, which constitutes about half the body-weight, had approximately one-fiftieth of the activity of the most active lymphatic tissue, and as most other tissues had activities of the order of one-tenth of the maximal lymphatic activity, one may conclude that not more than a thirty-thousandth of the dose lodged in the tissues.

If this represents the fraction traversing the wall of the alimentary tract, such traversal is clearly an improbable event. As such, it may still be important as a route of infection. But it seems very unlikely that a similar process could be of nutritional significance.

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WE are grateful to Prof. R. B. Fisher for his comments but must point out that no claim was made that "similar processes may be of nutritional significance". We presume the sentence under question is: "We suggest that the use of radioactive particles of this kind may prove to be a sensitive method for the study of various problems of infectious disease and possibly also in work on nutrition and nutritional allergy"; but this can scarcely be taken as implying that the absorption of particles is of nutritional significance.

It seemed to us that the demonstration of particulate absorption, however small, might be of interest to those working on the absorption of relatively insoluble elements required in only trace amounts. In this connexion it may be of interest to record that we are endeavouring to determine the relative uptake of particles of different sizes. Experiments of this kind, attempting to measure the 'porosity' of the alimentary canal, may well find some application in work on nutrition although we are mainly interested in the pathogenesis of infectious disease.

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HÆMATOLOGY

Antigenicity of A_2 Hæmoglobin

THE heterogeneity of adult human hæmoglobin is a well-established fact[1-3]. The present work was designed to determine if the slow electrophoretic fraction A_2 has antigenic specificity and if this fraction can be detected immunologically in cord blood and in the presence of hæmoglobin S and C. Previously described[4] techniques were used for the preparation and the agar-gel electrophoresis of the hæmolysates. Following electrophoresis the agar gel was cut into sections containing the hæmoglobin fractions to be tested for antigenicity. These sections were then frozen, thawed and filtered through fritted Büchner funnels of medium porosity. The short duration (90 min.) and the use of large glass plates as the base of the agar gel made it possible to obtain quantities of eluates sufficient for immunization of rabbits. Eluates of the following fractions were used for this purpose: the main component (A_1) of normal blood and of blood from a patient with thalassæmia minor; the respective A_2 fraction and the non-hæmoglobin fraction which we have previously[4] designated 'unidentified component 1' and which probably is identical with Derrien's X_1 fraction[2]. In addition the most cathodic part of the fœtal hæmoglobin from cord blood (Fig. 1*b*, area between brackets) was eluted from agar gel because of the possibility that this part contains A_2 hæmoglobin. Each eluate containing approximately 3 mgm. of protein was combined with an equal volume of Freund's adjuvant and injected into adult male albino rabbits on three or four different sites.

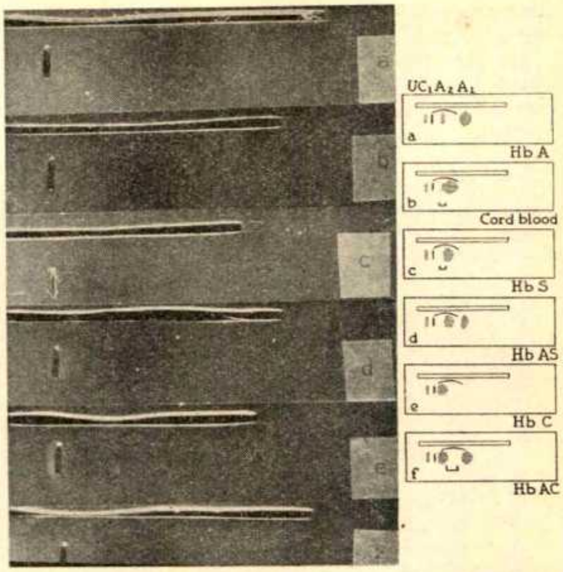

Fig. 1. Immuno-electrophoretic precipitin reaction of A_2-antiserum and hæmolysates. The brackets in *b*, *c*, *f* indicate the areas which were isolated and eluted for immunological studies

Twenty-one days following the injection blood was obtained from the ear artery and this procedure was repeated at weekly intervals. Serum from these samples was tested for antibody content by applying the Ouchterlony agar-gel diffusion technique as modified in this Laboratory[5] and immuno-electrophoresis in agar gel on microscope slides. As test antigens the following material was used: (*a*) agar-gel eluate of the A_1, A_2 and unidentified component 1 fractions of normal and thalassæmic hæmoglobin; (*b*) whole hæmolysates of normal adult and cord blood and of blood from patients with thalassæmia minor, sickle cell anæmia, sickle cell thalassæmia, A–S and A–C hæmoglobin; (*c*) eluates of sections of electrophoretograms of fœtal, S, and A–C hæmoglobin which correspond to the location of the A_2 fraction (Fig. 1*b*, *c*, *f*, areas between brackets).

Antibody to A_2 hæmoglobin appeared three weeks after a single injection of the A_2 fraction. This antibody reacted with hæmolysates of normal adult and fœtal hæmoglobin, hæmoglobin S, A–S, A–C (Fig. 1) and S thalassæmia. The precipitation line was always in a position opposite to a place on the electrophoretogram corresponding to the A_2 fraction. On Ouchterlony plates all hæmolysates reacted with the antiserum, but when eluates of the isolated fractions were used as test antigens, only A_2, the slowest section of S hæmoglobin, and the eluate of the interspace of A and C reacted, which are the areas corresponding to the location of the A_2 component. Antisera to the slowest section of fœtal hæmoglobin reacted in the same manner as the antiserum to A_2 from thalassæmic and normal blood, suggesting immunological identity of these fractions.

Antibody to A_1 and to unidentified component was demonstrated only following one or two booster injections. A_1 was only weakly, but the unidentified component 1 was strongly, antigenic (Fig. 2). Anti-unidentified component 1 serum cross-reacted slightly with A_2 but not with A_1. A clearly defined precipita-

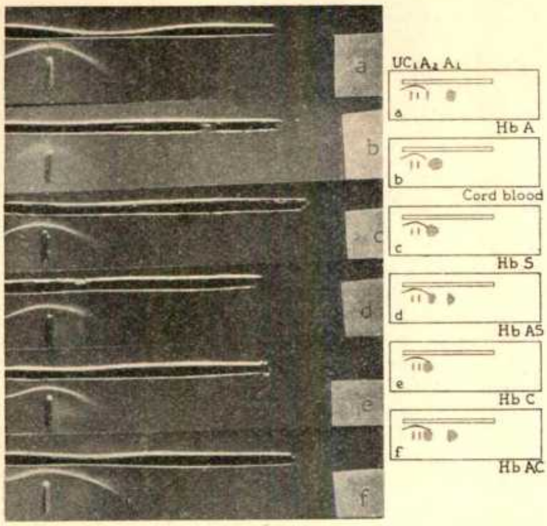

Fig. 2. Immuno-electrophoretic precipitin reaction of the unidentified component 1 antiserum and various hæmolysates

tion line was also present over the unidentified component 1 area in immuno-electrophoretograms of whole hæmolysates of normal adult, fœtal, S, A–S, C and A–C hæmoglobin (Fig. 2). With fœtal hæmoglobin (Fig. 2*b*) this line was not so strong as with the other hæmolysates. Anti-unidentified component 1 antibodies reacted slightly with γ-globulin of serum, but anti-γ-globulin did not react with the unidentified component 1.

It appears from the results of this work that A_2 hæmoglobin has specific antigenicity and that it can be immunologically demonstrated in hæmolysates of fœtal, sickle cell, and C hæmoglobin. Boivin and Hartman[6] recently reported that whole hæmolysates of fœtal, A, S, C and E hæmoglobin share at least two antigenic fractions one of which appears to be the slow component A_2. The present work lends support to this finding by the demonstration of the specific antigenicity of the isolated A_2 fraction. This finding is of interest in view of the recently detected differences in the 'finger print' between A_1 and A_2 hæmoglobin[7].

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Two New Hæmoglobin Variants in a Very Young Human Embryo

IN a previous communication[1] we reported the results of a study of the hæmoglobin of 26 human fœtuses and reviewed the relevant literature. In 25 fœtuses from pregnancies of 10 weeks or more we were unable to find a major hæmoglobin component which differed from fœtal hæmoglobin (Hb–F) or adult hæmoglobin (Hb–A). In a 9-week fœtus of 3·5 cm. crown–rump length, however, the largest hæmoglobin component was found to differ from Hb–F and Hb–A ; its rate of alkali denaturation was intermediate, as also was its electrophoretic mobility in agar at *p*H 6·2, and it was assumed to be the 'embryonic' hæmoglobin of Drescher and Künzer[2]. The present communication describes the finding of two new hæmoglobin variants in a human fœtus of only 3·4 cm. crown–rump length from a 10½-week pregnancy. For convenience these will be referred to as hæmoglobins Gower 1 and Gower 2.

Approximately 0·2 ml. of blood was obtained immediately after death from the umbilical cord of the intact embryo, following therapeutic termination of pregnancy by hysterotomy, and a hæmoglobin solution prepared from the washed red cells by the method of Singer *et al.*[3]. The behaviour on alkali denaturation and electrophoresis was studied within 24 hr. of the death of the fœtus and the ultra-violet absorption spectrum was analysed later. The results were compared with those obtained on hæmolysates of blood from older fœtuses, full-term new-born infants and adults.

The rate of alkali denaturation studied by the method of Künzer[4] fell within the range given by Drescher and Künzer[2] for cord blood from full-term infants, but outside our own narrower range[1]. The proportion of alkali-resistant hæmoglobin calculated from the rate curve was 56 per cent.

On electrophoresis in agar with citrate buffer *p*H 6·2 (ref. 5) two fractions separated, one migrating similarly to an Hb–F marker and the other to an Hb–A marker.

Electrophoresis on cellulose acetate both with barbiturate buffer *p*H 8·6 and with 0·04 *M* sodium phosphate buffer *p*H 7·7 separated three main fractions plus a very small fast component. The largest fraction corresponded to Hb–F. Hb–A, which can be separated from Hb–F under the particular conditions used with barbiturate buffer, was not detected.

With starch-gel electrophoresis in *tris*-citrate/borate discontinuous buffer[6] three fractions were separated (Fig. 1). The largest corresponded to Hb–F, while the second and third were shown to move more slowly than the Hb–C and Hb–G/C respectively of a hæmolysate containing Hb–A, Hb–G, Hb–C and Hb–G/C (ref. 7). Again no Hb–A appeared to be present.

Direct spectrographic examination of the separated zones in the starch gel was carried out after the gels had been made transparent with glycerol[8]. The tryptophan fine-structure band had the following positions on the fœtal fractions :

Major zone (Hb–F)	2899–2900 Å.
Gower 1 zone	2908 ± 2 Å.
Gower 2 zone	2902 ± 2 Å.

Values obtained for Hb–A and Hb–F zones in control starch-gel analyses of hæmolysates prepared from adults and new-born infants were as follows :

Hb–F	2900 ± 1 Å.
Hb–A	2910 ± 1 Å.

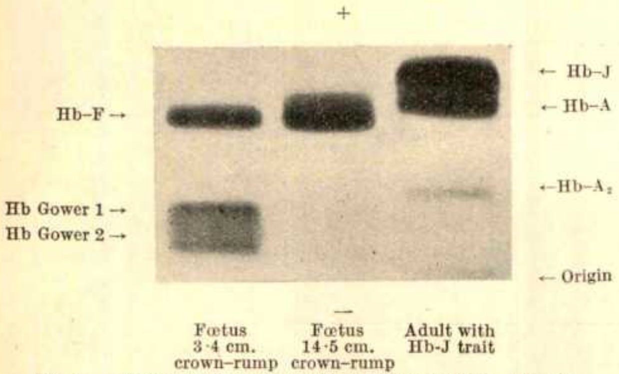

Fig. 1. Starch gel electrophoresis of hæmoglobin in the *tris*-citrate/borate discontinuous buffer system (initial gel *p*H 8·8)

The precision of the latter readings was superior as the hæmolysates were more concentrated, but the results on the fœtal blood suggest that spectrographically the hæmoglobin in the Gower 2 zone is of fœtal type whereas that in the Gower 1 zone resembles Hb–A. It should be noted that the position of the peak of the tryptophan fine-structure band for Hb–F differs from the figure of 2896–7 Å. previously reported[8,9]; this may be due to the presence of non-hæm proteins, some of which are known to migrate to the Hb–F position, as well as to background scatter contributed by the gel under the conditions used here. Non-hæm proteins may also have influenced the reading in the Gower 2 zone.

The above findings indicate that this embryo carried, in addition to Hb–F, three other hæmoglobins. The behaviour of the very minor fraction detected on cellulose acetate suggested that it could be one of the fast-moving hæmoglobins occasionally found in some cord bloods[10–12], but this hæmoglobin will not be discussed further because the minute amount of the specimen precluded adequate study. Although an appreciable fraction moved to the Hb–A position on agar electrophoresis, it could not in fact have been Hb–A because, by electrophoresis on cellulose acetate at *p*H 8·6 and on starch gel, no Hb–A was detected. Examination of the blood of both parents, who were of Jewish European stock, showed only normal hæmoglobin components by starch-gel electrophoresis and less than 2 per cent Hb–F by the 1-min. alkali denaturation procedure[3], so it is extremely unlikely that this fœtus was a carrier of a variant of Hb–A. The Gower 1 and Gower 2 fractions may be presumed, therefore, to represent variants of Hb–F or 'embryonic' hæmoglobin. Their behaviour on electrophoresis at alkaline *p*H distinguishes them from all the described Hb–F variants, for Hb–Alexandra[13] which alone moves more slowly than Hb–F differs by migrating faster than Hb–C. Neither can represent the Hb–F_I and Hb–F_{II} fractions separated by chromatography on 'Amberlite *IRC* 50' resin[14], because we have confirmed (authors' unpublished observations) that these column fractions are virtually indistinguishable from Hb–F both electrophoretically and spectrographically[15] and in their rate of alkali denaturation[15,16]. Further precise characterization of the fractions Gower 1 and Gower 2 was not possible, but our results indicate that neither can be as resistant to alkali as Hb–F and that Gower 2 is spectrographically related to Hb–F.

We think it probable that these two new hæmoglobins will rarely be seen since we have encountered them only once in the course of examining blood from 32 human fœtuses, and have not found a similar report in the literature. Moreover, we think that it may well be significant that the only two fœtuses in which we have seen hæmoglobins other than Hb–F, Hb–A and Hb–A_2 had crown–rump measurements of only 3·5 and 3·4 cm., and such small fœtuses are rarely available for study.

We wish to thank Mr. F. Ward for the photograph.

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PATHOLOGY

Tumour-Promotion by Lime Oil in the Mouse Forestomach

In a recent communication[1] it was shown that four different citrus oils promoted tumour development in mouse skin after pretreatment with a sub-carcinogenic dose of 9,10-dimethyl-1,2-benzanthracene (DMBA). Since man is continuously exposed to these oils in his food, it was decided to test one of them, lime oil, both for carcinogenicity and for tumour-promoting activity in the gastro-intestinal tract of mice. Another reason was the hope of demonstrating the two-stage mechanism of carcinogenesis in the alimentary canal. Previous attempts to do this have given inconclusive results[2,3]. In the most recent of these, Berenblum and Haran gave mice a single dose of one of four polycyclic hydrocarbons followed by 3 per cent croton oil weekly for 30 weeks. All treatments were given by stomach tube, and polyethylene glycol 400 (PEG) was the solvent throughout. The appropriate control groups were included. Numerous tumours of the forestomach developed in all their groups, both test and

Table 1. INCIDENCE OF TUMOURS OF THE FORESTOMACH

Group	Tumour-initiating treatment (a single dose given by stomach tube in 0·25 ml. PEG)	Tumour-promoting treatment (0·05 ml. given by stomach tube once weekly for 40 wk.)	Mice with tumours/ Survivors for 50–280 days*	Tumours > 3 mm. diameter	Total tumours	Average No. of tumours per mouse
I	100 μgm. DMBA	Lime oil	9/9	10	55	6·1
II	100 μgm. DMBA	None	8/10	1	27	2·7
III	200 μgm. 3,4-BP	Lime oil	10/11	8†	38	3·8
IV	200 μgm. 3,4-BP	None	5/11	1	14	1·3
V	50 μgm. 3,4-BP	Lime oil	8/9	3	20	2·2
VI	50 μgm. 3,4-BP	None	0/9	0	0	0
VII	12·5 μgm. 3,4-BP	Lime oil	4/9	2	9	1·0
VIII	12·5 μgm. 3,4-BP	None	2/10	0	2	0·2
IX	Solvent only	Lime oil	3/11	0	4	0·4
X	None	Lime oil	2/8	1	3	0·4
XI	None	None	0/9	0	0	0

* The remaining mice are still under observation.

† One squamous carcinoma invading muscle.

control. It was not stated whether the rest of the intestinal tract was examined. No evidence for tumour-promotion by croton oil was obtained. There are three possible reasons for this: (1) The doses of the hydrocarbons used were too high. Bock and King[4] have since shown that the mouse forestomach is more sensitive than the skin to carcinogenic hydrocarbons. (2) Croton oil, in the concentration used, was carcinogenic. (3) PEG, although a suitable solvent for a single dose of a hydrocarbon[5], reduces the tumour yield when given repeatedly after such a dose[3] and is not, therefore, a suitable solvent for tumour-promoting substances.

In the following experiment some of these difficulties have been overcome; and early results indicate a definite tumour-promoting effect.

Stock female albino mice were randomly divided into 11 groups of 20. Before each treatment the mice were starved for 18 hours. A single dose of DMBA or 3,4-benzpyrene(3,4-BP) in 0·25 ml. of PEG was given by stomach tube, followed, after an interval of 3 weeks, by 40 weekly doses of 0·05 ml. of undiluted lime oil by stomach tube. At this dose-level the mice remained healthy and maintained a steady body weight of 32–35 gm. Mice which died between the fiftieth and the two hundred and eightieth day were examined *postmortem*, and half the survivors at 280 days were killed and similarly examined. Details of treatment and the results are summarized in Table 1.

Tumours of the forestomach were seen in all the groups receiving DMBA or 3,4-BP except group VI, but in the corresponding groups treated with the same doses of DMBA or 3,4-BP, followed by lime oil, many more tumours developed. The difference was most clearly shown in the groups receiving 50 μgm. 3,4-BP. Higher doses of DMBA and 3,4-BP partially obscured this effect. When only the tumours more than 3 mm. in diameter are considered the difference is obvious in all groups: only 2 were seen in the controls, but 22 in the test groups. So far, one malignant tumour has been seen in group III. It was a squamous cell carcinoma, with an area of poorly differentiated cells infiltrating the muscle in several places. In the two groups (IX and X) which received lime oil, but no DMBA or 3,4-BP, 6 tumours were seen including one of 4 mm. diameter. No tumours have been seen in the completely untreated controls, nor in a larger number of untreated mice kept for general observation. It seems that both the hydrocarbons and lime oil possess some oncogenic effect, but the yield of tumours in the groups treated with both agents considerably exceeded the sum of their separate effects. No tumours were seen in the glandular part of the stomach, or elsewhere in the alimentary canal.

These results represent half the experimental mice; the remainder are still under observation, as it is probable that more malignant tumours will develop after 40 weeks, as in similar work on the skin[6].

While the evidence for a two-stage mechanism of carcinogenesis was limited almost entirely to studies on mouse and rabbit skin it was possible to suppose that it was peculiar to the skin and not involved in the genesis of tumours in other tissues. The demonstration of a two-stage process in the mouse forestomach increases the likelihood that it is a general phenomenon. It is true that the epidermis and the epithelium of the forestomach are both of squamous type, but they differ widely in function, in environments to which they are exposed, and in their apparent liability to neoplastic change.

Man is inevitably exposed to small quantities of 3,4-BP and other carcinogens in his environment. Although the amounts may be insufficient to account for carcinogenesis, they are theoretically sufficient for initiation. Recently Roe, Salaman and Cohen[7] have postulated that cigarette smoking acts mainly as a promoter in the induction of human bronchial carcinoma. It is possible that citrus oils act in a similar way in the causation of cancer of the upper alimentary tract. The dose given in this experiment, a total of 2 ml. per mouse during 40 weeks, is approximately equivalent to 4 litres ingested by a man over 30 years, on the basis of comparative body-weight and life-span, and may not be unrealistic. However, it is impossible, at this stage, to extrapolate with any confidence from mouse to man.

I wish to thank Dr. F. J. C. Roe and Dr. M. H. Salaman for their valuable advice throughout this experiment. The expenses of this research were partly defrayed out of a block grant from the British Empire Cancer Campaign.

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Acquired Porphyria in Man and Rat due to Hexachlorobenzene Intoxication

SINCE 1956, an outbreak of cutaneous porphyria involving several thousand cases has been noted in three south-eastern provinces of Turkey[1]. Affected individuals, predominantly children and adolescents, exhibit photosensitivity with hydroa æstivale, marked porphyrinuria and hepatomegaly, but no abdominal or neurological symptoms. Epidemiological studies suggested the possibility that the disease might be related to the ingestion of wheat treated with a fungicide containing hexachlorobenzene[1,2]. Since direct verification of this possibility by field investigation has not been obtained[1], and since the available toxicological information on hexachlorobenzene is conflicting[3], an attempt was made to produce hexachlorobenzene porphyria in rats.

Adult male Sprague-Dawley rats were fed *ad lib.* ground 'Purina Laboratory Chow' containing 0·2 per cent hexachlorobenzene. Urinary and fæcal excretion of porphyrins and porphyrin precursors were determined periodically[4], and tissue porphyrins[5] and liver catalase[6] were measured in rats killed while porphyric.

Of a total of 33 rats started on the experimental diet, 13 died within the first month, exhibiting terminal tremor, ataxia, weakness, and paralysis, without evidence of major disturbance in porphyrin metabolism. In the remaining 20 rats, a significant increase in urinary excretion of porphyrins and porphyrin precursors was first noted after 2 to 8 weeks of hexachlorobenzene administration (Fig. 1). Peak excretory values per 24 hr. were as follows (normal values in parentheses): δ-amino-lævulinic acid, 1·40 mgm. (0·06 mgm.[7]); porphobilinogen, 16·96 mgm. (0·01 mgm.[7]); uroporphyrin, 0·360 mgm. (trace[8]); coproporphyrin, 0·211 mgm. (0·016 mgm.[8]). Slight to moderate increases in fæcal excretion of copro- and proto-porphyrin were also noted. From 200 ml. of rat urine, 55 mgm. of crystalline porphobilinogen were obtained[9].

In rats in which hexachlorobenzene administration was discontinued shortly after these peak excretory values had been approximated, the disturbance in porphyrin metabolism was rapidly reversed with return to normal excretion values within a week. However, maintenance of high levels of porphyrin excretion by continued feeding of hexachlorobenzene for two to three weeks resulted in an apparently irreversible porphyric state, in which marked porphyrinuria continued in spite of cessation of drug administration (Fig. 1). These animals died with the neurological complications described above.

Hepatomegaly was common in porphyric rats. Histological studies revealed liver cell degeneration, most prominent around central veins, and increased size and number of Kupffer cells. Chemical analysis of liver tissue showed the following results, per 100 gm. wet tissue (normal values in parentheses): uroporphyrin, 0·4–0·5 mgm. (trace[8]); copro- and proto-porphyrin, 0·05–0·15 mgm. (0·01–0·02 mgm.[8]); porphobilinogen demonstrable[5] in aqueous extract of liver tissue. No significant reduction in liver catalase activity was found.

In the blood, concentrations of hæmoglobin, copro- and proto-porphyrin were normal, while uroporphyrin was not detectable. At autopsy, intense red fluorescence was observed in the cortex of long bones, but not in the marrow.

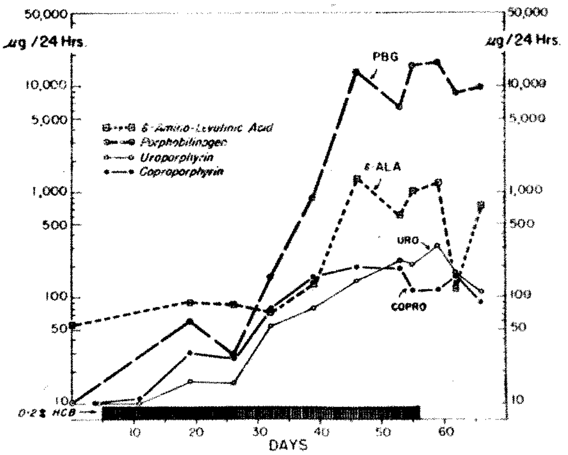

Fig. 1. Urinary excretion of porphyrins and porphyrin precursors in a 350-gm. male rat fed hexachlorobenzene. The rat died on the 68th day of the experiment, that is, 63 days after the start of the hexachlorobenzene administration and 12 days after the toxin had been withdrawn

These results demonstrate that in the rat chronic ingestion of hexachlorobenzene may indeed result in a profound disturbance in porphyrin metabolism, characterized by increased amounts of porphyrins and porphyrin precursors in the liver and excreta, with histological evidence of hepatocellular degeneration. These findings support the suggestion[1], derived on the basis of epidemiological data, that hexachlorobenzene ingestion is the cause of the recent outbreak of porphyria in Turkey. This experimental result is of particular interest in that, hitherto, human porphyria was assumed to be genetically determined, because conclusive evidence for the existence of a purely acquired form of this disease has been lacking. The widespread appearance of porphyria among three genetically distinct populations in south-eastern Turkey—domestic Turks, Kurds and Turks repatriated after several centuries of residence in the Balkans—makes its dependence on an inherited abnormality extremely unlikely.

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RADIOBIOLOGY

Autoradiographical Detection of Tritium-labelled Inulin in the Kidney

By perfusion tests performed on the kidneys of rabbits which had previously been intravenously injected with inulin (100 mgm./kgm. body-weight), it has been shown that inulin was deposited in the kidney tubule cells[1]. These observations were recently confirmed by Balint and Forgacs[2]. Other authors, however, did not agree with my conclusions[3,4].

The autoradiographical detection method seemed suitable to us to obtain further information about the distribution pattern of inulin in the kidney. By means of this technique using tritium-labelled inulin we investigated the kidneys of rats and rabbits.

Alkali-stable inulin was labelled with tritium gas according to the method of Wilzbach[5] (Graul and Hundeshagen[6]). This preparation was dissolved in water and evaporated three times. The final product was obtained by crystallization from an aqueous solution with a mixture of ethanol–ether (v/v, 1/1). Its specific activity was 71·5 μc./mgm. or 286 c./mol. (assumed molecular weight: 4,000), and was alkali-resistant like the initial product. In solution the substance obtained gave a negative reduction test, and after hydrochloric hydrolysis it gave a positive Selivanoff reaction. This is sufficient guarantee that the inulin molecule had not been altered by labelling.

Five mgm. of tritiated inulin was injected under direct vision into the renal artery of an anæsthetized rabbit. After 5 min. the kidneys were removed and fixed in formalin. Transverse sections were examined by the autoradiographical method[6], using an exposure time of 2–9 months. After developing the photochemical layer, the tissue sections were stained with hæmatoxylin–eosin.

Figs. 1 and 2 represent autoradiograms obtained. Over the cells of the proximal and distal tubules there is an obvious blackening, which in other fields appears still more intense, and is even visible in some instances over the tubule lumina.

We obtained corresponding results in rats injected intravenously with tritiated inulin (50 mgm./kgm. body-weight). In these experiments the kidneys were removed after 10 and 20 min. The autoradiograms were not as intensively marked as in the above-mentioned tests, but generally a blackening over the tubule cells was clearly visible.

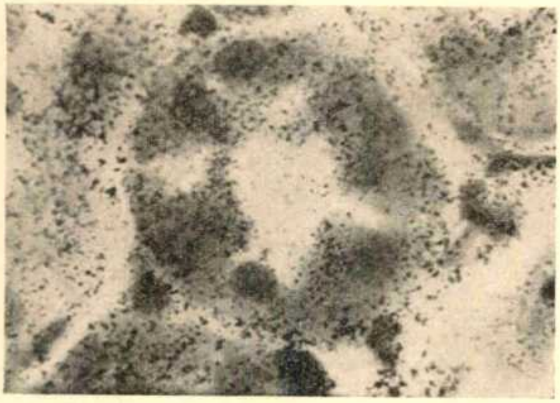

Fig. 1. Autoradiogram of a rabbit kidney 5 min. after injection of 5 mgm. tritiated inulin into the renal artery. Exposure time, 6 months. Proximal tubule. (× c. 900)

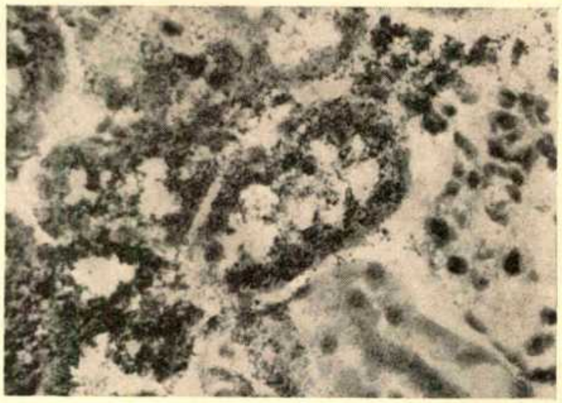

Fig. 2. Experiment similar to Fig. 1. Distal tubule, same as Fig. 1. (× c. 360)

The results of these autoradiographical investigations on kidneys with tritiated inulin confirm our opinion that inulin is deposited in the tubule cells of the kidney. No information about the mechanism of this process was obtained.

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Experimental Allergic Encephalomyelitis in Irradiated Rats

Lipton and Freund[1] have produced experimental allergic encephalomyelitis in the rat by intracutaneous injection of homogenized homologous rat brain mixed with paraffin oil and tubercle bacilli. Lumsden[2] reported earlier that in the rat experimental allergic encephalomyelitis can be induced only by injecting the antigen mixture using the intracutaneous and not the subcutaneous route.

If the concept of radiation sickness as an auto-immune disease[3] is valid, then the question may be put whether it would be possible to induce experimental allergic encephalomyelitis in irradiated rats.

In the concept of auto-immune reactions of irradiated animals, which is being reported elsewhere[3], it is necessary to presume that antibody-producing cells undergo a change, that is, they mutate, thus becoming foreign to their own organism. In that manner these cells become antigenically stimulated by the organism's own cells—both normal and those which had also undergone a mutation. Antigenically stimulated cells should be radio-resistant, since immunity already acquired is not impaired by irradiation. By absorbing radiant energy, the antibody-producing cells mutate until they acquire a structure which, according to the clonal selection theory[4], corresponds to the antigens present in the body. From that moment they should behave as 'already antigenically stimulated cells', that is, they will be radio-resistant and will proliferate.

Experiments were planned so that during irradiation the reinforced brain antigen was already present in the body. The antigen was injected into experimental animals shortly prior to irradiation, with the intention that it should become attached to mutated antibody-producing cells and thus stop their further mutation and direct the immune reaction towards the recipient's own neural tissue.

Twenty albino rats were divided into two groups. The first group, consisting of ten animals, was injected subcutaneously with 2 × 0·25 ml. per animal of Freund's mixture of homologous rat brain and adjuvant; the second group of ten animals was given 4 × 0·25 ml. per animal. The amount of tubercle bacilli in the adjuvant was ten times less than prescribed by Freund in order to determine whether irradiation exerts any enhancing effect on the development of the auto-immune process. For the same reason the otherwise inadequate subcutaneous route of injection was used. The injections were given 30 min. prior to X-irradiation. The dose was 600 r. (220 kV., 15 m.amp., 0·5 copper, 1·0 aluminium, r = 40 cm. ∼ 120 r./min.). The control group consisted of ten unirradiated animals divided into two groups of five animals each and were injected with the same antigen doses and by the same route.

Results are presented in Table 1. The diagnosis of experimental allergic encephalomyelitis was based on paralysis of limbs and/or marked atactic gait. Eight out of twenty irradiated animals have shown these symptoms, which appeared between the thirteenth and the twentieth day following irradiation. Two belonged to the group which received 2 × 0·25 ml. of antigen mixture, and six to the group which was given 4 × 0·25 ml.

Table 1. INCIDENCE OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN UNIRRADIATED AND IRRADIATED RATS FOLLOWING SUBCUTANEOUS INJECTION OF ANTIGEN REINFORCED WITH ADJUVANT

Group of rats	No. of rats	No. of rats which developed symptoms of EAE
Unirradiated	10	0/10
Irradiated	20	8/20

It can be concluded that the inactivity of the immune system following irradiation is only apparent. It is supposed that radiant energy induces mutation of cells which belong to the immune system so that it becomes foreign to its own organism. The mutated antibody-producing cells might react immunologically against any antigen already present in the body and particularly against the antigen reinforced by adjuvant. In order to avoid the objection that the animals were irradiated during the inductive phase, another group consisting of 50 albino rats was injected with the antigen mixture 30 min.–24 hr. following irradiation. The incidence of experimental allergic encephalomyelitis in this group was 19 : 50.

This result favours the concept that the auto-immune processes had already started at the moment of irradiation and that antigen injection had directed it against the recipient's own neural tissue. Although this result requires further investigations, it does not invalidate essentially the concept of the auto-immune process as a cause of radiation sickness.

The beneficial effect of isologous bone marrow in the treatment of irradiation sickness might represent a serious objection to the concept of irradiation sickness as auto-immune disease. It should, however, be kept in mind that by injection of bone marrow we carry into the irradiated animal normal cells capable of antibody production.

The possibility that increased permeability of cells caused by irradiation might accelerate the contact of antigen with antibody-producing cells would mean that histamine or serotonin injection should enhance the auto-immune reaction. However, these two compounds are potent protectors against radiation damage.

Analogous experiments using other organs as antigen and changing dose and time of injection and dose of irradiation should elucidate this phenomenon and check the validity of the concept.

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Distribution of Phenyl Mercuric Chloride labelled with Mercury-203, applied as a Seed Dressing, in the Tissues of the Young Carnation Plant

ORGANO-MERCURY compounds are good fungicides; they are applied to oat seeds to control *Helminthosporium* infection[1], to *Narcissus* bulbs to control *Fusarium oxysporum*[2] rot and to gladiolus corms to prevent disease caused by *Sclerotina gladioli*[3]. However, the mercury compound may damage the plant; growth of oat seedlings is less than normal when phenyl mercuric acetate is applied to the soil to protect seed[4]. Experiments described here illustrate the way in which damage to seedlings may occur when phenyl mercuric chloride is used as seed dressing to control rot in carnation seedlings.

Seeds of Annual Charm carnations were treated with varying amounts of phenyl mercuric chloride labelled with mercury-203 as follows. Groups of twenty seeds were placed in Petri dishes on wire gauze supports over a solution of phenyl mercuric chloride in dioxan (2 mgm./ml.). Control groups were exposed to dioxan vapour only or left in the dishes in the same place as experimental seeds. The amounts of dressing on the seeds were varied thus: (*a*) by exposing seeds to the vapour from the phenyl mercuric chloride solution for 24 hr. at 18° C. (Exp. 1); (*b*) by exposing them for 2–3 days at 37° C. (Exps. 2, 3); (*c*) by dipping them in the dressing solution and drying with filter paper (Exp. 4). The amount of phenyl mercuric chloride on the seeds was measured in a well-type gamma-scintillation counter which had an efficiency of *c*. 34 per cent for mercury-203. The seeds were sown in crystallizing dishes containing sterilized vermiculite, horticultural grade. They were watered with a solution containing 5 gm. urea, 5 gm. potassium dihydrogen phosphate, 2 gm. magnesium sulphate, 1 gm. ferrous sulphate and traces of manganese sulphate, calcium chloride, boric acid

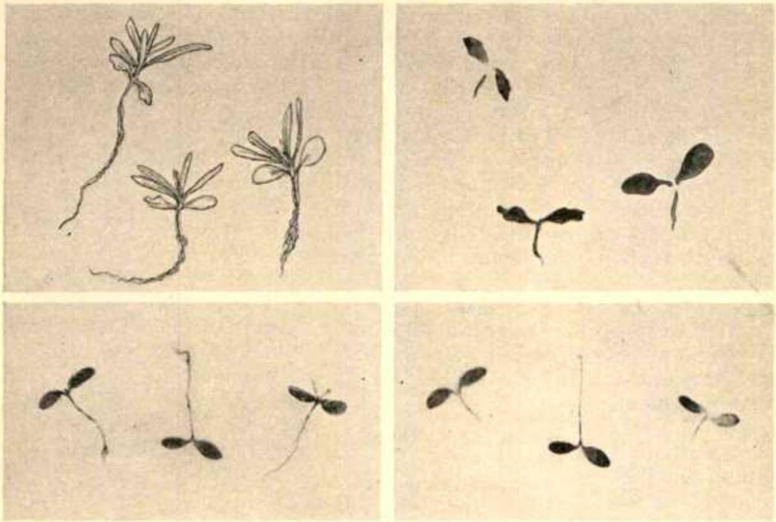

Fig. 1. Top, Exp. No. 2. Left, 8-week-old seedlings; right, autoradiograph of the seedlings to show the distribution of phenyl mercuric chloride labelled with mercury-203. Bottom, Exp. No. 3. Left, 7-week-old seedlings; right, autoradiograph to show the distribution of the labelled phenyl mercuric chloride

and sodium molybdate dissolved in 1 gal. water. The dishes were covered with polythene sheets and placed in 30° C. incubator until germination occurred. After about 3–4 weeks the seedlings were transplanted into larger enamel trays containing vermiculite and nutrient solution. The testas from germinated seeds and 4 young plants from each experiment were counted. Results are tabulated in Table 1.

The treated seedlings from Exps. 1 and 2 grew well, but in 1, one seedling had mottled cotyledons, and in 2, three seedlings had this appearance. In Exp. 3, four seedlings died early. All these failed to develop a normal root and one had mottled cotyledons. None of the control plants had damaged roots or cotyledons. In Exp. 4 the seeds received a dose of β-radiation in the range of 17–22 rads when they took 7 days to germinate. This is probably insufficient to reduce germination, since a dose of $3 \cdot 6 \times 10^3$ rads is necessary to reduce the germination of very radiosensitive seeds of *Pinus sylvestris* by 50 per cent (H. J. Bowen, private communication). However, when the seeds were treated with inactive phenyl mercuric chloride so that approximately 200 µgm. of material was deposited on the seed, germination was completely inhibited.

Young plants from experiments 1–3 aged eight or nine weeks were taken from vermiculite media washed to free the roots from particles, dried and pressed on to 'Cellophane'. They were then exposed to 'Ilfex–X-ray' film for 4–6 weeks and the film developed normally.

Table 1

Amount of activity, counts/min.		Weight (av.) of phenyl mercuric chloride/ seed (µgm.)	Testa counts/ min. (av.)	Seed-ling counts/ min. (av.)	No. of seedlings appeared	
Exp. No.	Av./seed				Treated with phenyl mercuric chloride	Dioxan control
1	978	1·92	236	284	15	16
2	1,997	3·9	330	244	13	11
3	3,663	7·23	502	1,144	14 (4 died)	11
4	95,050	186·6	no germination		—	13

In Exp. 1, 17 out of 20 untreated seeds germinated.

Fig. 1 shows the distribution of radioactivity in the plant. Phenyl mercuric chloride has concentrated strongly in the cotyledon leaves, hypocotyl and root adjacent to the hypocotyl. These plant organs appeared to be damaged in some plants in the experiments described. Some seed leaves contained phenyl mercuric chloride but were not mottled, and only a proportion of the roots which contained the compound were damaged.

An experiment was made to discover whether the smallest amount (1·92 µgm.) of phenyl mercuric chloride on the seed would prevent an infection of Dianthus seeds caused by *Trichothecium*. Seeds which had small fungal colonies on the testa were placed over phenylmercuric chloride in dioxan (2 mgm./ml.) for 24 hr. at room temperature. This treatment was the same as that in Exp. 1, which resulted in 1·92 µgm. of labelled phenyl mercuric chloride being deposited on the seed. Thirty treated seeds and untreated seeds were placed on moist tissue paper to germinate. After four days the untreated seeds were covered with mould and all failed to germinate. 75 per cent of the treated seeds germinated; only one showed mould, which was a species of *Penicillium*.

One of us (P. F.) has observed damage similar to that caused by phenyl mercuric chloride to the seedlings of carnations when seed was treated with 'Vegetable Seed Saver'. The cotyledon leaves were spotted with areas of dead tissue and growth was retarded. Occasionally seedlings failed to grow at all because no root developed. 'Vegetable Seed Saver' contains the fungicide tetra methyl thiuram disulphide (thiram). The damage caused by this and phenyl mercuric chloride is very similar with this strain of carnation.

Phenyl mercuric chloride has a strong affinity for the sulphydryl groups of protein[5]. The above results suggest that phenyl mercuric chloride has combined with protein substances in the cotyledons, hypocotyl and root, organs which are recognizable in the seed. The mercury protein was transferred from the hypocotyl to the root, but the amount in the root tip was less than that in the region nearest the hypocotyl (Fig. 1). This distribution could be explained by assuming that the mercury-protein is transferred passively during cell division and that new protein is being actively synthesized in the region of the root tip. The new protein would dilute the mercury-protein present in the dividing cell. The —SH-containing protein necessary for root growth could be derived initially from S—S protein in the cotyledons and later by synthesis from sulphate present in the growth medium. No transfer of mercury protein to the stem apex or leaves occurs although large amounts are present in the cotyledons; the reason for this is not apparent.

The similarities between the damage caused by phenyl mercuric chloride and tetra methyl thiuram disulphide may be the result of coupling with the same sulphydryl-containing compounds. Certain disulphides are known to couple selectively with —SH-containing compounds. However, a more direct comparison of the action of these fungicides on plant tissue could be made if thiram was labelled with sulphur-35. The experiments do indicate that phenyl mercuric chloride applied at the rate of 2 μgm./seed is an effective fungicide. In amounts greater than 4 μgm./seed the seedlings will be damaged, probably owing to the reduction in the quantity of SH-protein in the root and cotyledon tissues.

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BIOLOGY

Species-characterizing Antigens of 'L' and 'ERK' Cells

CELL culture has become a very important procedure in both biological and medical research, and with the many continuous lines of cells available to-day it is necessary to have methods to check the identity of any given culture of cells.

With proper attention to the special needs of the problem, there must be many immunological methods that could be adapted for this purpose. Recently, we[1] have reported on the use of the mixed agglutination reaction for the recognition of the species of origin of cells in culture. In this communication we wish to report briefly our findings on two continuous lines of cells, namely, the 'L' and 'ERK' strains, using the mixed agglutination reaction and antisera apparently specific for tissue cells of the various animal species.

The histories of these two strains are as follows. The 'L' strain[2] is of mouse origin, the parent strain having been derived in 1940 from the subcutaneous and adipose tissue of a 100-day-old male mouse of the *C3H* strain Andervont sub-strain. One group of cultures from this parent strain was treated with 3-methylcholanthrene at a concentration of 2·0–2·5 μgm./ml. for 111 days, and was designated strain 'L'. The parent strain prior to this treatment was grown on a fowl plasma–embryo extract clot, with a supernatant fluid medium consisting of Earle's saline (40 per cent), horse serum (40 per cent) and fowl embryo extract (20 per cent); strain 'L' cells were cultured initially in the same way, and have since been grown as monolayers on glass under the same fluid medium for some years. The cells used here were derived from a sample provided by Dr. J. Paul, and have been grown as monolayers on glass in Eagle's medium supplemented with 10 per cent bovine serum.

The 'ERK/1' strain was derived by trypsinization of one kidney of an embryonic rabbit by Westwood *et al.*[3]; it was cultured initially on glass in roller bottles in a medium consisting of rabbit serum (10 per cent), 'TPY' supplement (5 per cent), cystein-hydrochloride (0·02 per cent) and Earle's saline (to

Table 1. MIXED AGGLUTINATION TESTS ON 'L' AND 'ERK' CELLS TO REVEAL THEIR SPECIES-CHARACTERIZING ANTIGENS

Red cell antisera rendered specific for recognition of species antigens on tissue cells	Indicator red cells	Cells growing in culture										
		Under test 'L' strain[1]	Control panel									Under test 'ERK'[11] strain
			Mouse[2]	Rat[3]	Bovine[4]	Pig[5]	Guinea[6] pig	Rabbit[7]	Rhesus[8] monkey	Human[9] *KB* strain	Human[10] HeLa strain	
Rabbit anti-human red cell[13]	Human group O	0	0		0	0	0	0	20	**160**	**160**	160
Guinea pig anti-rabbit red cell[13]	Rabbit[12]	0	0	0	0	0	0	**80**	0	0	0	0
Rabbit anti-pig red cell[14]	Pig group O	0	0	0	0	**20**		0			0	0
Rabbit anti-bovine red cell[13]	Bovine[12]	0	0	0	**640**	0	0	0	0	0	0	0
Rabbit anti-mouse red cell[13]	Mouse	320	**320**	80	0	0		0	0	*	0*	0*

Figures in columns indicate titres of serum giving mixed agglutination. Where no figures are shown no test was performed. Homologous reaction in bold type.

[1] Cultured, from sample provided by Dr. J. Paul, in Eagle's medium supplemented with 10 per cent bovine serum.
[2] Isolated by tryptic digestion from new-born mouse skin; cultured in medium 199 supplemented with 20 per cent mouse serum.
[3] Isolated by tryptic digestion from fœtal rat skin; cultured in medium 199 supplemented with 20 per cent rat serum.
[4] Isolated by tryptic digestion from adult bovine kidney; cultured in mixture of 79 per cent Hanks's solution, 20 per cent bovine serum, 1 per cent lactalbumin hydrolysate in P.B.S.
[5] Isolated by tryptic digestion from adult pig kidney; cultured in mixture of 79 per cent Hanks's solution, 20 per cent pig serum, 1 per cent lactalbumin hydrolysate in P.B.S.
[6] Isolated by tryptic digestion from adult guinea pig kidney; cultured in medium 199 supplemented with 20 per cent guinea pig serum.
[7] Isolated by tryptic digestion from adult rabbit kidney; cultured in medium 199 supplemented with 20 per cent rabbit serum.
[8] Isolated by tryptic digestion from adult rhesus monkey kidney; cultured in medium 199 supplemented with 0·14 per cent bovine albumin and 0·1 per cent peptone.
[9] Derived from a sample obtained from the Research Institute (Animal Virus Diseases), Pirbright; cultured in Eagle's medium supplemented with 10 per cent bovine serum.
[10] Derived from a sample obtained from the Institute for Medical Research, Mill Hill; cultured in Eagle's medium supplemented with 10 per cent bovine serum.
[11] A sample obtained direct from Dr. J. C. N. Westwood (cultured in Earle's medium supplemented with 15 per cent calf serum).
[12] Red cells treated with papain before being used in the tests.
[13] 1 : 10 most concentrated dilution of serum tested.
[14] 1 : 5 most concentrated dilution of serum tested.

* Occasionally slight affinity between cultured cells (untreated with specific antiserum) and mouse red cells—a phenomenon that is being investigated.

100 per cent) ('TPY': Tryptic meat broth, 88 per cent; peptic digest of sheep's blood, 2 per cent; yeast extract, 10 per cent). Epithelial cells grew out first, but were later replaced by fibroblasts, which persisted until the seventh passage. At this time most of the cells degenerated, and the cultures were kept for 17 days without a change of medium. A plaque of epithelial cells was then noted in one bottle, and from this plaque the strain 'ERK/1' was easily developed. The sample of 'ERK/1' cells used here was provided by Dr. J. C. N. Westwood and had been cultured in Earle's medium supplemented with 15 per cent calf serum.

The mixed agglutination reaction was developed by Coombs *et al.*[4] for the purpose, originally, of demonstrating blood group antigens on tissue cells. The reaction involves the formation of mixed clumps (which are easy to observe under a phase-contrast microscope) between tissue cells and indicator red cells if the two cell types possess a common antigen.

The adaptation of the method to the problem of recognizing the species-antigenic characteristics of cells growing in culture depends on the assumption, upheld empirically[1], that red blood cells and other tissue cells of the body, or such cells in culture, possess in common certain antigens characterizing the species. In the tests made so far, anti-red cell sera have been used and have been absorbed with tissue cells and red cells of various heterologous species to render them species-specific in their reaction[1]. In essence the reaction is performed as follows. The suspension of cultured cells under investigation is treated in separate tests with the diagnostic red cell antisera, and after being washed free of uncombined antibody is added to a suspension of homologous red cells. An affinity between the antibody-treated tissue cells and the red cells, manifested by the formation of mixed agglutination, indicates common antigenic structures and by implication the species nature of the test cell.

Subjecting 'L' and 'ERK' cells, together with a large panel of cultured cells of known origin as controls, to such an examination gave the results shown in Table 1; relevant details about each cell culture are given in a footnote to Table 1.

The specificity of the diagnostic sera used may be seen from the results of the tests on the control panel of cultured cells. There seems to be no doubt that 'L' cells are still mouse in antigenic character, since they reacted strongly with the anti-mouse serum and not at all with the sera diagnostic for the human or bovine species or that of the rabbit and pig. On the other hand, the 'ERK' cells failed to give a positive reaction with the anti-rabbit serum but reacted strongly with the anti-human serum only. The most obvious conclusion is that the 'ERK' cell is a human cell.

Other characterizing antigens may be used in such mixed agglutination tests on cultured cells besides these rather ill-defined 'species antigens'. Tests for human *iso*antigens have already been reported on HeLa cells[5], and our findings will soon be published on the use of 'heterophile' antibodies establishing the presence of antigens of the Forssman type.

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Nucleic Acids as Transplantation Antigens

Much has been written about the role of nucleic acids in transplantation immunity[1-3]. From previous work it has been concluded that deoxyribonucleic acid (DNA) was not antigenic. The following results, based on chemical analysis including chromatography of sugars[4] and of purines and pyrimidines or their derivatives, suggest that ribonucleic acid (RNA) also is not a necessary part of the antigenic molecule. Antigens from mouse spleen and thymus were prepared by different procedures[5-7]. Their antigenicity was checked by techniques previously described[3].

Antigen I has been prepared by precipitation at pH 5 (Table 1). Accordingly, its RNA content was high. By incubation with trypsin, 60–80 per cent of this material was solubilized. The sediment (preparation II) kept the major part of the antigenic activity, its RNA content was considerably lowered, while its percentage in galactose + mannose was increased. Further treatment of this sediment by ribonuclease led to the removal of any detectable trace of RNA without any loss of activity (preparation III). By this treatment no change in the hexose content was found.

It must be emphasized that preparation I, not previously treated by trypsin, was resistant to ribonuclease, the RNA fraction being most probably surrounded by a protective proteic layer. The constant hexose content at about 3–4 per cent, coupled with a percentage of hexosamine to proteins at 1–2

Table 1. Composition in Percentage of some Transplantation Antigens extracted by Different Procedures

	Injected dose (mgm. of proteins)*	Proteins	DNA	RNA	Galactose + mannose
I (ref.5)	1·5	83·3	1·1	13·9	1·7
II†	0·5	92·6	3	2·48	4·9
III‡	0·5	96		0	4
IV§ (ref.6)	0·22	93·5	2·5	1·5	2·5
V	1·1	67·1	29·3	1·5	3
VI	0·15	85·1		11·8	3·1

* Usually equivalent to 500 × 10^6 cells; preparation V from 5×10^9 cells.
† Sediment after centrifuging 30 min. at 25,000*g* the trypsin-treated preparation I.
‡ Sediment after centrifuging 20 min. at 25,000*g* the ribonuclease-treated preparation II.
§ Kindly supplied by Dr. G. Lejeune.

per cent, suggests that the antigenic determinant could be carried by a glycoprotein. It must be added that the presence of fucose is suspected but could not be confirmed. Rhamnose was never found. Table 1 includes the analysis of other preparations obtained by different procedures. Preparation IV[6] is, in part, based on the action of trypsin. Its composition is very near that of preparation II. Preparation V is that of Billingham, Brent and Medawar[7]; it is very rich in DNA, but its hexose content is very similar to that of previous preparations. Preparation VI[8] has been obtained by electrophoretic separation.

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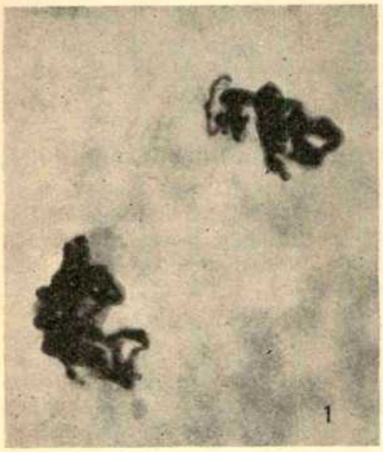
Electron Microscopic Autoradiography in a Magnetic Field

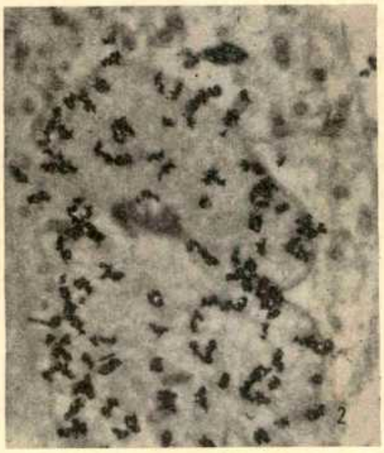
THE feasibility of electron microscopic autoradiography was suggested to us by the report of O'Brien and George[1]. We have tried a similar technique with tritiated thymidine in tissue culture and have found that recognition of reacted grains in the nuclear track emulsion may be greatly facilitated by their distinctive filamentous appearance, and that the intensity of labelling may be increased by exposing the grids in a strong magnetic field.

HeLa cells were grown in a medium containing tritiated thymidine, and standard techniques were used to prepare thin sections of the cells for electron microscopy. A small amount of Kodak nuclear track emulsion *NTB*3 was liquefied by heating in a water-bath at 45° C. and was diluted with 2 parts of water. After stirring, the diluted emulsion was allowed to stand for 20 min. at room temperature to eliminate bubbles. Sections mounted on stainless steel grids were touched to drops of the diluted emulsion, and the excess emulsion was drained away by touching the edges of the grids to filter paper. After exposure in the dark for several weeks and developing with *D*19 developer, examination in the electron microscope showed filamentous spots over the nuclei of cells (Fig. 1). Such spots were shown to be the result of radioactivity by controls in which the sections of cells were omitted and the tritiated compound was mixed with the emulsion. That the spots consisted of reacted grains in the emulsion was demonstrated when no radioactive substance was present and the grids were exposed to light.

Some reacted grains were visualized outside the nuclear membrane, and we sought to eliminate this scatter by exposure of the emulsion-covered grids to a magnetic field. The use of a magnetic field was suggested by Dr. M. Ter Pogossian, who calculated that the low-energy electrons of tritium would be affected by a flux density of 10 kilogauss and who was able to supply a magnet of this intensity. The result did not show complete elimination of scatter, and it was difficult to make any quantitative estimation of the amount of scatter. However, it was found that the reacted grains over nuclei in preparations exposed to the magnetic field were considerably more numerous than in preparations not exposed (Figs. 2 and 3).

It appears that the distinctive contour of the filamentous spots and the increased intensity of labelling brought about by the magnetic field are technical aids which may help to combine the advantages of tritium autoradiography with the resolving power of the electron microscope. The filamentous shape of the reacted grains may be used to distinguish them from other spots and from arte-





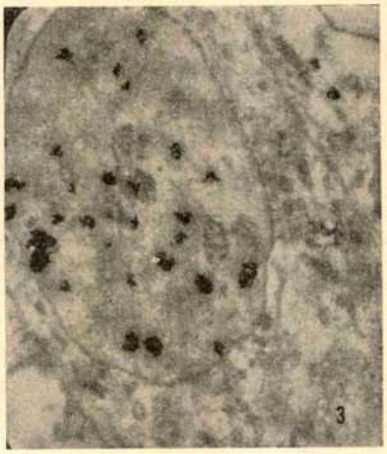


Fig. 1. Filamentous contour of reacted grains over the nucleus of a HeLa cell. The nuclear track emulsion was exposed to the section for 8 days. (× 37,500)

Fig. 2. Reacted grains over the nucleus of a cell after exposure of the emulsion to the section for 32 days in a magnetic field. (× 3,500)

Fig. 3. Control emulsion exposed to the section for 35 days without a magnetic field. (× 3,500)
Cells were grown for 2 days in the medium containing tritiated thymidine (3·3 μc. per ml. of medium, specific activity 890 mc. per m.mole)
Sections for Figs. 2 and 3 were cut from the same block and were slightly thicker than those usually used for electron microscopy

facts that may be seen in electron micrographs. The increased numbers of reacted grains after exposure of emulsion-covered grids to a magnetic field may serve to shorten the times needed for exposure and reduce the amount of radioactivity needed in thin sections to obtain satisfactory electron autoradiographs.

A more detailed account of this work will be published in *Laboratory Investigation*.

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Status of the Grey Seal

THE leading article in *Nature* on "The Grey Seal and British Fisheries"[1], by relying on Rae's[2] appendix, gives a wrong impression of our knowledge of the status of the grey seal (*Halichoerus grypus*) in British waters both now and in the past. There has in fact at no time been adequate information on which to base a reliable estimate of the total population of this species. Added to this ignorance there has been, and still remains, much confusion with the common seal (*Phoca vitulina*), and the recent work of several zoologists on the biology of the grey seal has resulted in the discovery of previously unknown breeding groups which are not necessarily new colonies. An increase in knowledge of a species does not mean that there has been an increase in its numbers.

Without direct total or sample counts neither absolute nor comparative populations can be estimated except by recourse to indirect methods. The biology of the grey seal creates difficulties at all stages; for example, although the breeding season extends for about three months, the breeding cows are present only for two to three weeks, so that on no one day can they all be counted together; some have departed before others arrive. The same applies to the pups, although they are present on the average for 4–5 weeks. The number of breeding bulls is much smaller, possibly as little as one-tenth that of the cows. Does this represent all the bulls of comparable ages with the cows, or are the others alive but at sea? Again the non-breeding individuals are conspicuous by their absence[3–5]. They must be at sea or in some unknown proportion hauled out on rocks which are not used for breeding.

At other times of the year the grey seals are distributed over fairly wide areas of the seas around the coasts of Britain, as marking investigations have shown. No counts are possible under these conditions.

To make a reasonable estimate of the population, it is therefore necessary to have some idea of a life-table. Davies[6] attempted this, but, where no information was available, had to make assumptions such as the age of puberty in both cows and bulls, and the potential longevity. Annual mortality-rates were taken from Kenyon and Scheffer for the northern fur-seal. Since Hewer[7] has shown how to determine the age of a grey seal, we now know that Davies's assumptions were incorrect. The cows do not live for twelve years but for more than thirty. They do not have their first pup when two years old but when about six. Such knowledge materially affects any calculation of population numbers. The position of the bulls is still obscure; but I am informed that there may well be fewer than half as many bulls as cows in any colony, taking non-breeding and breeding individuals together. This alone would reduce the estimated population by 25 per cent.

The moral of this surely is that we should forget all previous guesses and figures since they cannot do more than lead us astray. Instead of arguing on the basis of valueless data, our efforts should be directed towards getting the data needed for reasonably sound estimates.

Meanwhile it should be remembered that even if the total number of grey seals is two or three times as great as the wildest estimate so far made, it would still be a comparatively rare seal on a world scale. The Norwegian catch of young harp and bearded seals combined (which is less than 50 per cent of the total catch) is about 300,000 per annum in recent years. Such figures could only be maintained on populations vastly in excess of that of the grey seal.

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Root-knot Nematodes and Legume Nodules

PUBLISHED descriptions of the course of infestation of roots of legume species by root-knot nematodes (*Meloidogyne* spp.) are not new. As early as 1932 Godfrey and Oliveira[1] followed the process of infestation in cowpea (*Vigna sinensis*) in root observation boxes containing sterilized soil.

In the Leguminosae, where the phenomenon of symbiotic nitrogen fixation is so significant, little appears to have been done in investigating the inter-relationships between *Rhizobium* spp., root-knot nematodes and their leguminous hosts, either under experimental or natural conditions. There is a mere mention by Van Schreven[2] and Masefield[3] regarding the possible role of the nematodes in modifying nodulation and nitrogen assimilation. Christie[4] reported finding root-knot nematodes in the nodules of soybean plants that were otherwise apparently free from nematode galls.

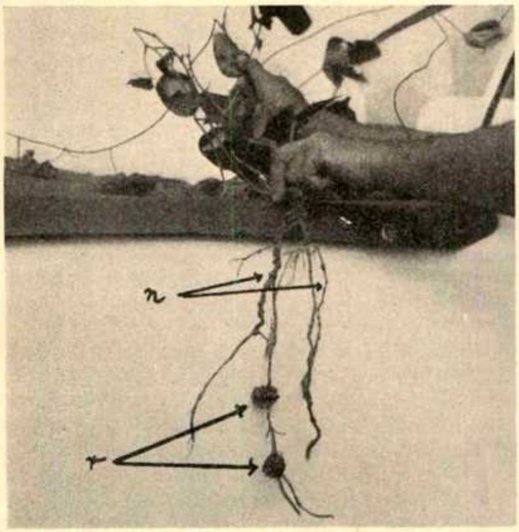

Fig. 1. Morphological difference between mature nematode-free nodules (*r*) and the gall-like structures (*n*) which may be either transformed nodules or true nematode galls, as found on velvet bean (*Stizolobium* sp.)

Recent field and laboratory observations of the root systems of the cowpea–legume group (*Vigna sinensis* var. and *Stizolobium* spp.) grown in sub-tropical and tropical locations of the sugar cane belt in Australia show that: (1) the infestation of nodules (and other root sites) of the above legumes by the root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood, is a common occurrence in these localities; (2) on certain plants the nodules only may be attacked; (3) throughout one growth-cycle a large percentage of nodules may become infested; solitary nodules in lower root zones will often escape infestation; (4) nodules reach various sizes before initial infestation though smaller (younger ?) nodules appear more susceptible.

The finding of nodules in all intermediate stages from those exhibiting larval penetration only (together with a preponderance of *Rhizobium*-occupied tissue) to those in which multiple or secondary infestation (but no bacteria) predominates suggests that the production of nodule or gall depends on the subsequent development of the early nodule after attack by the eelworm (Fig. 1). It is not yet known whether nodules can resist or recover from nematode infestation, and answers to these questions are being sought from present work under controlled conditions.

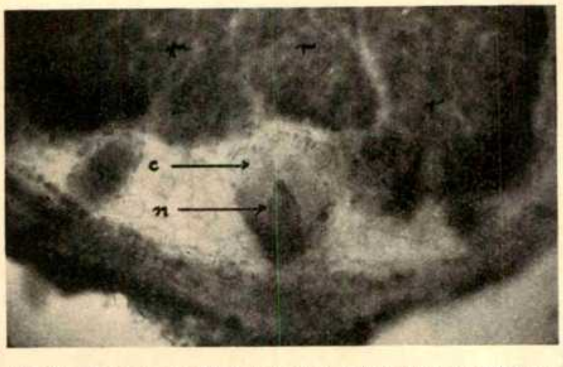

Fig. 2. Transverse section through a nodule from velvet bean showing (*r*) inner tissue containing *Rhizobium*-filled cells, (*n*) sessile female *Meloidogyne* developing in the outer cortex, behind which the outlines of giant multinucleate cells may be seen (*c*). (× *c*. 56)

One interesting feature of the secondary infestation of the nodule by the nematode is that giant cells develop on which the swollen female nematode feeds, as in the case when plant roots are carrying true nematode galls (Fig. 2). This raises the intriguing speculation as to whether a third member (the nematode) may be added to the physiologically specialized relationship which now exists between legume host and *Rhizobium* spp.

In so far as the cowpea–legume group is concerned, the nematode–nodule association is sufficiently widespread to encourage inquiry into its effects on: (*a*) the establishment and growth of the cowpea species used for green manuring throughout the Australian sugar cane belt; (*b*) the symbiotic nitrogen assimilation of such crops. These matters will continue to be investigated at the Macknade Experiment Station in North Queensland.

I thank Dr. J. B. Goodey, Rothamsted Experimental Station, for identifying the root-knot nematode.

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ENTOMOLOGY

Toxic Hazard from formulating the Insecticide Dimethoate in Methyl 'Cellosolve'

DIMETHOATE (O,O - dimethyl S - (N - methylcarbamoylmethyl) phosphorodithioate) is an effective systemic insecticide for use on plants[1-3], has shown promise as a chemotherapeutic agent for control of cattle grubs[4,5] and certain other insect pests of farm animals, and has high contact toxicity to house-flies and many other insects. The pure material, with a rat oral *LD*50 of about 600 mgm./kgm., is the least toxic to mammals of the systemic insecticides at present available for use in controlling plant pests. Commercial dimethoate is 2–4 times more toxic to rats than the pure compound, and repeated recrystallization or chromatography is required to achieve the reduced mammalian toxicity of the pure material. Several investigations have been reported on the chemical[6] and biological[7-9] degradation of dimethoate.

During the purification of commercial dimethoate, a fraction was separated which potentiated the rat oral toxicity of pure dimethoate. Addition of 2–10 per cent of this fraction to pure dimethoate potentiated its toxicity (rat oral *LD*50) by 3–4 times, a finding which at least partially explains the difference in toxicity between the pure and commercial material. This fraction also potentiated the toxicity of malathion and O,O-dimethyl S-carbamoylmethyl phosphorodithioate to the rat. The potentiator appears to be effective only with certain species, since the commercial material was more toxic than the pure compound to rats, guinea pigs, rabbits and hens, but not to mice, pheasants, locusts, aphids and flies. With rats the potentiator was active by the oral and intra-peritoneal routes but not following dermal administration. On demethylation of dimethoate by reaction with salts of O,O-dimethyl phosphorodithioic acid, O,O,S-trimethyl phosphorodithioate and a potentiating material were formed. The potentiator, also formed by the reaction of dimethoate with lithium chloride, may be a salt of O-methyl S-(N-methylcarbamoylmethyl) phosphorothioic acid or a closely related compound. On analogy with the potentiation of pure dimethoate by O-ethyl O-*p*-nitrophenyl phenylphosphonothionate, tri-*o*-cresyl phosphate and tri-*o*-cresyl phosphorothionate[10], it would appear that the potentiating impurity in commercial dimethoate might act by reducing the efficiency of the dimethoate detoxification mechanism acting through hydrolysis of the carboxyamide grouping.

Methyl and ethyl 'Cellosolve' (2-methoxy- and 2-ethoxyethanol) have excellent solvent characteristics for the formulation of organophosphorus insecticides, particularly where a high degree of systemic activity is desired after application to foliage. As a result of routine toxicity checks on the properties of technical dimethoate formulated in methyl 'Cellosolve', an unexpected toxic hazard was observed. The rat oral *LD*50 decreased on storage from an initial 150–250 mgm./kgm. to 30–40 mgm./kgm. after seven months storage in England. After nine months storage under tropical conditions the *LD*50 was less than 15 mgm./kgm., and after storage at 100° C. for 63 hr. in the laboratory the *LD*50 was 8 mgm./kgm. The mammalian toxicity increased several-fold before any change in insecticidal activity was evident. Further development of this unstable formulation was immediately stopped.

Chromatography by described procedures[7,8] of the decomposed dimethoate in methyl 'Cellosolve' yielded fourteen phosphorus-containing products. The degradation involved hydrolysis at the amide and all the ester groupings, a marked loss of thiono sulphur, and replacement of both the O-methyl and N-methylamino groupings by 2-methoxyethanol. No evidence was obtained for the formation of pyrophosphates or thionopyrophosphates. The most toxic fraction was purified and identified as an O,O-dialkyl S-(N-methylcarbamoylmethyl) phosphorothiolate with probably one, but possibly both, methyl groups of dimethoate replaced by 2-methoxyethyl groupings. This compound was less toxic to flies than dimethoate, but had a rat oral *LD*50 of about 1 mgm./kgm. and a mouse intra-peritoneal *LD*50 of about 0·5 mgm./kgm. The increase in toxicity to mammals of the decomposed dimethoate formulation was greater than that attributable solely to this single toxic product. Since no other single component was toxic enough to account for the discrepancy, it appears that a potentiation of toxicity also occurred among the materials in the dimethoate - methyl 'Cellosolve' formulation.

The relationship of stability to toxicity of dimethoate formulated in a number of solvents was investigated. Little if any hydrolysis or change in mammalian toxicity occurred in non-hydroxylic solvents. Polyhydroxylic solvents resulted in stability during short storage without the formation of highly toxic degradation products. Of several monohydroxylic solvents, only methyl and ethyl 'Cellosolves' resulted in formulations highly toxic to mammals on short-term storage at 70° C. From this work a commercial formulation was developed which showed no significant change of toxicity after 162 days storage at 40° C.

The potential hazard which could have arisen is not unique for dimethoate, since O,O-dimethyl S-(N,N-dimethylcarbamoylmethyl) phosphorodithioate, O,O-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate (malathion), O-methyl O-(2,4,5-trichlorophenyl) phosphoramidothionate (Dow *ET*-15) and O,O-dimethyl O-(2,4,5-trichlorophenyl) phosphorothionate (Dow *ET*-57) were also found to show a several-fold increase in mammalian toxicity after reaction with methyl 'Cellosolve' at 70° C. for eight days.

When 'Cellosolves' are to be used in formulating phosphorothionate insecticides, the possible ester and amide exchange reactions to yield products of increased mammalian toxicity should be carefully investigated. A more complete account of these investigations will appear elsewhere.

Publication of this communication is approved by the Director of the Wisconsin Agricultural Experiment Station, and by the Directors of Fisons Pest Control, Ltd. The investigation was supported in part by a Haight Travel Fellowship from the Graduate School of the University of Wisconsin to one of us (J. E. C.), and by grants from the U.S. Public Health Service, National Institutes of Health, and from the U.S. Atomic Energy Commission (Contract No. *AT*(11–1)–64, project No. 14). We thank Dr. E. F. Edson, Dr. G. S. Hartley, Mr. A. J. Lambie and Mr. P. Carter of Fisons Pest Control, Ltd., and Mrs. Lydia McBride of the University of Wisconsin, for their encouragement and assistance.

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Lysine as a Mosquito Attractant

PREVIOUS work has indicated that attractive factors other than carbon dioxide are present in the vapour from mammalian blood[1] and body exudations[2]. A distillate obtained from mammalian blood proved highly attractive to *Culex pipiens*[3]. It has been recently reported that a mixture of nine biological acids and bases was attractive to *C. pipiens* and

Anopheles maculipennis at concentrations down to 0·05 per cent, although none of the compounds was attractive when tested individually[4].

Our experiments on *Aedes aegypti* were guided by an old report[5] that peptone and certain amino-acids had shown attractiveness for *A. sollicitans*, and by the fact that protein hydrolysates are employed successfully as baits for fruit flies. Control and experimental solutions were exposed on filter-paper in warm Petri dishes[1] in a cage containing mosquitoes maintained only on sugar-water; the number of approaches to the experimental solution, as compared to that to the control, was expressed as the attractiveness ratio. Of 16 different protein hydrolysates commercially available and tested in 10 per cent solution, 11 proved to be attractive. Of these, 8 were more attractive than ox blood plasma; on distilling them to dryness at 7 mm. mercury pressure and 37° C., the distillates of five of them proved significantly attractive.

Individual amino-acids were then tested, dissolved in distilled water in the relative amounts in which they occur in 10 per cent lactalbumin hydrolysate. Of the 17 tested, the only amino-acids to be significantly attractive were (with their attractiveness ratios): L-arginine 1·24, L-alanine 1·32, and L-lysine 3·84. A mixture of all the amino-acids except lysine showed an attractiveness ratio of 1·14; addition of lysine raised the attractiveness ratio to 2·80. At the buffered *p*H of 7·4, lysine showed an attractiveness ratio of 3·13, and raised the attractiveness ratio of the amino-acid mixture from 1·28 to 6·13.

L-Lysine hydrochloride was not attractive in acid solution, but became highly attractive in alkali (attractiveness ratio 2·59 at *p*H 7·4); L-lysine free base was attractive also in acid solution (attractiveness ratio 5·62 at *p*H 4·4). The hydrochloride of the unnatural enantiomorph D-lysine showed an attractiveness of 1·20 at *p*H 6·5 and of 3·70 at *p*H 11·6. The potency of L-lysine at *p*H 10 was such that in 0·001 per cent and 0·0001 per cent (1 p.p.m.) solutions its attractiveness ratios were respectively 5·95 and 2·86. When a 0·7 per cent solution of L-lysine was distilled at 7 mm. mercury pressure and 37° C., the distillate showed an attractiveness ratio of 7·73 and gave a positive ninhydrin test.

When a mixture of 0·88 per cent L-lysine and 0·24 per cent L-alanine was chromatographed through a 50 × 1·2 cm. column of 'Dowex 50*W*-*X*4' resin[6] and the successive eluate fractions tested, the attractiveness of alanine appeared at 40–50 ml. and that of lysine at 75–88 ml. after the addition of the sample to the column. When deproteinized lactalbumin hydrolysate (10 and 0·1 per cent solutions assayed) was similarly fractionated, the greatest attraction was at 66–90 ml., with a minor peak at 53 ml. The fraction at 87 ml. was tested by paper chromatography and found to contain L-lysine. When deproteinized ox blood plasma was chromatographed, the main peak of attractiveness was around 90 ml., with a smaller peak at 30–50 ml.; the amino-acid present in the 93- and 88-ml. fractions was identified as L-lysine. When deproteinized human blood plasma was fractionated, the main peaks were at 40–50 ml. and around 80 ml.; the fractions at 44 and 81 ml. were found to contain L-alanine and L-lysine respectively. In all the fractionations, a minor peak was found at about 120 ml., possibly due to L-arginine.

The results thus indicate that the most attractive principle in the protein hydrolysate and blood plasmas tested was L-lysine. Of possible contaminants, ornithine and asparagine showed no attractiveness, whereas ammonia, putrescine and cadaverine were strongly repellent at high concentrations and never became attractive on progressive dilution. L-Lysine proved attractive not only to the Orlando strain of *Aedes aegypti*, but also to strains from Penang, Key West and Trinidad. It was also attractive to field-caught *Aedes stimulans*, and to *Culex pipiens* at evening. A solution of L-lysine exposed in the cage stimulates the resting mosquitoes to take flight, and they react to it as to a guinea pig or human hand. The concentration of L-lysine in human blood[7] and sweat[8] is about 27 μgm. per ml. Since it may be distilled from aqueous solution and is attractive at 1 p.p.m., L-lysine could thus be the main attractive factor of mammalian blood and body emanations.

Full details of the work will be published elsewhere. We are indebted to the Defence Research Board of Canada for financial support of these investigations.

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A Method of dissolving the Protective Spittle Masses of Frog Hopper Nymphs

DURING the course of biochemical investigations into the nature and composition of the spittle masses produced by cercopid nymphs (*Philaenus spumarius* L. and *Neophilaenus* sp.) it was observed that these masses decomposed within a few minutes of being placed in a 2/3 *N* solution of sulphuric acid.

Since Cercopidae are of considerable economic inportance, especially *Aeneolamia varia saccharina* in the cane sugar industry, and are protected from the action of insecticides by the spittle which they produce, it was realized that the above observation might be utilized in the measures taken for their control.

It was shown in the laboratory that the rate of solution of spittle masses (Fig. 1) was dependent on the normality but largely independent of the nature of the acid, hydrochloric and nitric being as effective as sulphuric acid. Since the rate of solution is not only dependent on the normality of the acid, but also on size of spittle mass and amount of agitation, the values given in Fig. 1 therefore relate only to spittle masses of the size produced by late fourth and early fifth instar nymphs, and subjected to gentle agitation. Field trials were then carried out using sulphuric acid at various concentrations. This acid was chosen because of its low vapour pressure. Dieldrin (0·06 per cent), added as Shell 'Dieldrex', was added to each solution to kill exposed nymphs and thus prevent the production of new spittle masses. The results are given in Table 1, which also shows the effect of weather on both the rate and total destruction of spittle masses.

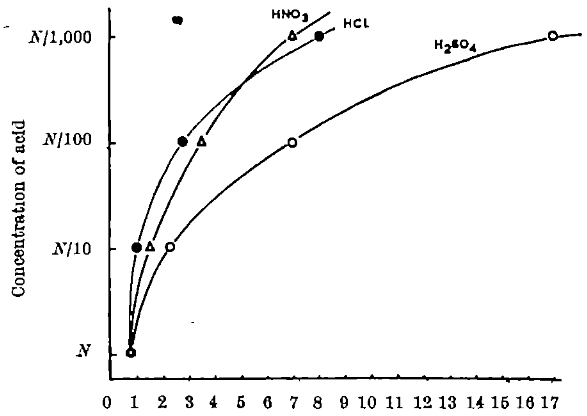

Fig 1. Rate of solution of spittle by acids

The phytotoxic effects of sulphuric acid on maize and oats, when sprayed as above at concentrations up to 2 *N*, were found to be negligible. Scorching of the leaves was produced by *N* and 2 *N* acid sprayed on at the rate of 100 gal./acre, but not by solutions of *N*/5 and *N*/10 acid. In neither case were subsequent growth and flowering affected by this treatment.

Similar results were found for grasses (species as in Table 1) although when using *N* acid the degree of scorching was distinctly greater. The use of *N* acid alone in this test resulted in a 95 per cent destruction of spittle masses within 48 hr.

Table 1. PERCENTAGE DESTRUCTION OF SPITTLE MASSES BY SULPHURIC ACID

Plots of grasses, each containing *Arrhenatherum elatius*, *Deschampsia caespitosa*, *Agrostis tenuis* and *Holcus lanatus*, were sprayed with solutions of sulphuric acid or water each containing 0 06 per cent dieldrin.

Test A. Carried out during a period of showery weather (total for the 3 days of the test. rainfall, 0 29 in.; sunshine, 15 5 hr.) on 4½ sq. yd. plots sprayed at the rate of 110 gall/acre.

Time (days)	Percentage destruction of spittle masses 1	2	3
N/5+0 06 per cent dieldrin	50 (41)	65 (59)	65 (62)
N/10+0 06 per cent dieldrin	38 (27)	59 (52)	62 (51)
Control (distilled water + 0 06 per cent dieldrin)	15	15	8

Corrected percentage destruction in parenthesis.

Test B. Carried out during a period of dry sunny weather (total for the 8 days of the test rainfall, 0 01 in.; sunshine, 17 6 hr.) on 5 sq. yd plots sprayed at the rate of 100 gall /acre:

Time (days)	Percentage destruction of spittle masses 1	2	3
N/5+0 06 per cent dieldrin	21 (28)	58 (62)	81 (81)
N/10+0 06 per cent dieldrin	38 (43)	51 (55)	85 (85)
N/50+0 06 per cent dieldrin	14 (21)	22 (28)	51 (51)
Distilled water+0·06 per cent dieldrin	0 (8)	3 (11)	30 (30)
Control untreated	−9	−9	0

Corrected percentage destruction in parenthesis.

These preliminary results therefore suggest that if sulphuric acid is added to solutions of acid-stable insecticides to give a final concentration of *N*/5, the nymphal mortality of Cercopids will be greatly increased. Using higher concentrations on crops not damaged by *N* and 2 *N* acid, an even greater mortality of nymphs can be achieved.

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BACTERIOLOGY

Microbial Production of Carbon Monoxide from Flavonoids

Aspergillus flavus degrades rutin to protocatechuic acid, phloroglucinol carboxylic acid, rutinose and carbon monoxide, the last-named being derived from carbon 3 of the flavonoid molecule[1,2]. The extent of distribution of this degradative pathway in micro-organisms and the structural requirements for the production of carbon monoxide were determined by comparing the amount of carbon monoxide produced after the growth of micro-organisms on various flavonoid compounds. The cultures studied were grown in sealed flasks of known volume[3]. A medium containing *N*—*Z* amine[1] was used when the substrates tested could be analysed by the aluminium chloride reaction. A synthetic medium[4] was employed with substrates that were difficult to determine directly and the amount of oxygen consumed was therefore used as the criterion of substrate utilization. Carbon monoxide and oxygen were determined by gas phase chromatography using a molecular sieve column.

The production of carbon monoxide from flavonoids is a common reaction in moulds but is absent in the bacteria tested (Table 1). As the recoveries of carbon monoxide are in general less than theoretical for the amount of substrate metabolized, there must be more than one pathway available to moulds for growth on flavonoid compounds.

A hydroxyl group in the 3-position is required for carbon 3 to be released as carbon monoxide (Table 2). Production of carbon monoxide is unaffected by the degree of saturation existing between carbons 2 and 3, or by the presence of a glycosidic bond in the 3-position (Tables 1 and 2).

Table 1. PRODUCTION OF CARBON MONOXIDE FROM CULTURES GROWN ON RUTIN, QUERCITRIN AND QUERCETIN

Culture	Rutin		Quercitrin		Quercetin	
	Per cent theoretical CO‡	Per cent substrate used	Per cent theoretical CO‡	Per cent substrate used	Per cent theoretical CO‡	Per cent substrate used
Alternaria sp.	78	91	43	7	18	30
*Aspergillus flavus**	94	100	34	6	35	40
*Aspergillus niger**	87	96	70	46	17	51
Cephalosporium sp.	82	95	106	98	63	30
Diaporthe sp	84	94	91	94	82	82
Fusarium sp †	87	100	106	97	95	49
Penicillium sp †	76	92	96	63	53	74
Pullularia sp.	82	99	88	25	16	44
Pseudomonas sp *	0	98	0	62	0	40

* Representative of results for 3 strains.
† Representative of results for 2 strains.
‡ Calculated on the amount of substrate used

Table 2. EFFECT OF SUBSTITUTION PATTERN OF FLAVONOID COMPOUNDS ON THE PRODUCTION OF CARBON MONOXIDE BY *Aspergillus flavus* PRL 1805

Substitution pattern	Δ-2,3 (flavone)	Δ-2,3; 3-OH (flavonol)	3-OH (flavanonol)	Nil (flavanone)
5,7,3′,4′-(OH)$_4$	—	+ (quercetin)	+ (taxifolin)	—
5,7.4′-(OH)$_3$	0 (apigenin)	+ (kaempferol)	—	0 (naringenin)
7,3′,4′-(OH)$_3$	—	+ (fisetin)	+ (fustin)	—
5,7,2′,4′-(OH)$_4$	—	+ (morin)	—	—
5,7,3-(OH)$_3$-4′(OCH$_3$)	—	—	—	0 (hesperetin)

—, Not tested.
+, Carbon monoxide production.
0, Growth, but no carbon monoxide produced.

Table 3. EFFECT OF SUBSTITUTION PATTERN OF FLAVONOID COMPOUNDS ON GROWTH OF TWO FUNGI AS INDICATED BY OXYGEN UTILIZATION

Class	Compound	Substitution pattern	Percentage of oxygen used	
			PRL 1805	PRL 30*
Flavonol	Quercetin	3,5,7,3′,4′-$(OH)_5$	25·4	43·6
,,	Morin	3,5,7,2′,4′-$(OH)_5$	4·1	2·4
,,	Kaempferol	3,5,7,4′-$(OH)_4$	55·1	55·3
,,	Fisetin	3,7,3′,4′-$(OH)_4$	10·5	6·3
,,	Robinetin	3,7,3′,4′,5′-$(OH)_5$	14·4	—
	Rhamnetin	3,5,3′,4′-$(OH)_4$-7(OCH_3)	26·6	—
	Azaleatin	3,7,3′,4′-$(OH)_4$-5-(OCH_3)	2·6	—
,,	—	3-(OH)-5,7,3′,4′-$(OCH_3)_4$	2·6	—
,,	—	5-(OH)-3,7,3′,4′-$(OCH_3)_4$	7·2	—
Flavanonol	Taxifolin	3,5,7,3′,4′-$(OH)_5$-2,3-$(H)_2$	36	—
Flavone	Apigenin	5,7,4′-$(OH)_3$	38·8	39·8
Flavanone	Naringenin	5,7,4′-$(OH)_3$-2,3-$(H)_2$	63·1	—
Flavanol	Catechin	3,5,7.3′,4′-pentahydroxyflavan	12·6	6·9

* *Fusarium* sp.

A. flavus cannot readily utilize quercitrin (quercetin-3-rhamnoside), but does utilize the aglycone; quercetin was obtained from quercitrin by acid hydrolysis. This implies that the production of the glycosidase which splits the rhamnosidic bond is the factor limiting growth on this substrate. It would also appear that the production of this enzyme limits the growth of the *Alternaria* sp. studied on quercitrin.

The effect of a 3-hydroxyl group on the point of cleavage of flavonoid compounds has been noted by Booth *et al.*[5]. These authors found that mammalian systems degraded flavonoid compounds with a 3-hydroxyl group to yield a phenolic acid with a 2-carbon side-chain, while those without this group yielded a phenolic acid with a 3-carbon side-chain.

The position of the substituent groups (that is, hydroxyls and methoxy groups) does affect the utilization of flavonoid compounds by micro-organisms (Table 3). For example, these cultures did not readily utilize morin as a carbon source but did use quercetin and kaempferol. It can be shown by use of molecular models that this is a result of the steric hindrance of the 3-position by the presence of a 2′-hydroxyl group. This steric hindrance implies a change in the electron distribution pattern around carbons 2 and 3 and/or hydrogen bonding between the 2′- and the 3-hydroxyl groups. The 4-position is implicated as being an active centre in the enzymatic attack of flavonoids as catechin was not readily utilized as a carbon source. Similarly, the 5-position is implicated by the low utilization of fisetin, robinetin and azaleatin. As apigenin, naringenin and taxifolin were readily utilized as carbon sources, neither a double bond between carbons 2 and 3 nor a hydroxyl group in the 3-position is required for ready utilization of the flavonoid molecule. The presence of methoxy groups on the flavonoid molecule interferes with the utilization of these compounds as carbon sources by the micro-organisms studied. However, the presence of a single methoxy group in the 7-position is without effect.

Hattori and Noguchi[6] reported the isolation of the depside of phloroglucinol carboxylic acid and protocatechuic acid after the growth of *Pullularia fermentans* var. *candida* on rutin. As the pathway outlined by our work[1,2] results in a similar product, it is quite likely that the carbon monoxide-producing pathway was used by *P. fermentans* during growth on rutin.

Micro-organisms[7], plants[7] and animals[8] have been reported to produce carbon monoxide. This communication reveals that flavonoid compounds are a potential source for carbon monoxide production by moulds. These facts, together with reports that micro-organisms are known which oxidize carbon monoxide[9], suggest that carbon monoxide is an important, active, 1-carbon metabolite in the carbon cycle.

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Examination of *Leptospira pomona* by Gel Diffusion

IN recent years, diffusible antigens of many bacteria and viruses have been demonstrated by the double-diffusion precipitation reaction in agar. Except for the work of Rothstein and Hiatt[1], who used the Oudin[2] technique in the study of chemically prepared fractions, we do not know of any investigation involving application of these methods in the study of *Leptospira*. This communication describes the results of an antigenic analysis of the four sub-serotypes of *Leptospira pomona*, attempted by diffusion of antigens and antibodies in agar.

Employing Mansi's[3] modification of Ouchterlony's[4] technique and hyperimmune sera prepared in rabbits, one common major line of precipitation was noted between antigen and antibody reservoirs when four antigenic sub-types were reciprocally examined against each other. However, differences in the line patterns were detected in the homologous systems; strains *pomona*, *C–B* and *C*-164 giving two reaction zones and strains *S*-91 and *G*-6 (Pande and Sekariah, 1960 [5]) producing only one line. These findings were confirmed with absorbed sera.

When fractions 1, 2 3 and 4 prepared from these sub-types, on the lines of Schneider[6], were tested against the homologous whole-cell antisera, a single line was detected with fractions 1 and 2 but no line with fractions 3 and 4, indicating that perhaps the latter antigens were located deep in the cell. Further tests showed that fractions 1, 3 and 4 were common to all sub-types within this serotype, while fraction 2 appeared to determine differences in serological reactivity.

When immune rabbit serum was examined against infected tissues, such as saline extracts of kidney lesions, a strong reaction was seen, whereas no reaction occurred between normal rabbit serum and infected tissues or suspensions and extracts of *Leptospira*. It has been found that 5 per cent formalin and 50 per cent glycerine do not destroy the antigen, and the test can be carried out satisfactorily on infected tissues preserved with these reagents.

It is intended to use this method in investigating field specimens obtained from suspected cases of leptospirosis in domesticated animals in India.

This test has also been successfully applied to detect antibodies in suspected sera when positive specimens were found to produce a single line of reaction within 2–3 hr. in the slide modification of the gel-diffusion test[7]. Precipitins could be detected by a comparable titre in the plate test. Further investigation is in progress to evaluate the diagnostic sensitivity and reliability of this method, on a comparative basis, the results of which will be reported in detail elsewhere.

We are indebted to Col. M. B. Starnes and Mr. A. D. Alexander, Division of Veterinary Medicine, Walter Reed Army Institute of Research, Washington 12, D.C., who kindly supplied the four sub-types of *L. pomona* and the homologous antisera.

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Localization of a Protease in the Cell of *Escherichia coli*

The cells of *Escherichia coli* contain a protease during their whole growth-cycle. This protease is inactive in living cells and its presence can be demonstrated only after disruption of the cell structure[1]. In connexion with the study of the function of this protease in the metabolism of intracellular proteins its localization in the cell was also investigated.

Spheroplasts were prepared from a 4-hr. culture of *E. coli B* by Lederberg's method[2] using penicillin. 20–30 per cent of the protease activity was transferred to the medium during the preparation of the spheroplasts. Because a part of the spheroplasts was disintegrated and their cellular proteins made soluble at the same time, it can be presumed that a greater part of the enzyme thus liberated is of intracellular origin. The spheroplasts were disrupted by osmotic shock in 0·01 *M tris* at *p*H 8·0 with 5×10^{-3} *M* magnesium chloride. The material was then centrifuged in the cold at 10,000*g* for 10 min. in an MSE centrifuge, the sediment washed with the buffer and again spun down. The combined supernatants were centrifuged for 1 hr. in a cooled Phywe ultracentrifuge at 105,000*g*, the pellet resuspended in the buffer and again spun down. The sediment at 105,000*g* exhibited a characteristic absorption at 257 mμ. The protease activity was determined with casein labelled with iodine-131 substrate[3] and referred to mgm. of protein, on one hand, and expressed as per cent of the whole amount of the enzyme, on the other. A loss of about 20 per cent of the enzymatic activity occurred during these operations.

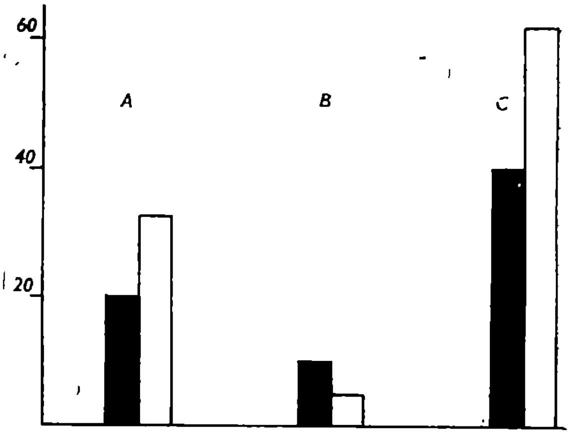

Fig. 1. Localization of a protease in cellular fractions of *E. coli*. *A*, Cytoplasmic membrane fraction; *B*, ribosome fraction; *C*, cytoplasm. Black columns, activity in μgm. of casein hydrolysed by 1 mgm. of protein during 1 hr. at 37° C.; white columns, percentage of total amount of protease

It was found that the enzyme is present mainly in the cytoplasm and in the fraction which sediments at 10,000*g* and contains the cytoplasmic membrane and remains of the cell wall. Only a very small part of the whole amount of enzyme was present in the fraction of ribosomes which sedimented at 105,000*g* (Fig. 1). Enzymatic activity in the ribosome fraction probably is not due only to contamination, because the protease activity, related to mgm. of proteins, amounts in this fraction to about one-half of that of cytoplasmic membrane and cannot be completely removed by washing. A certain amount of active protease present in the ribosome fraction in the *E. coli* cell is analogous to the localization of the peptidase[5]. The ribosomes of *E. coli* also contain a ribonuclease[6] which is present in an inactive form and is activated after the disruption of ribosomes by ethylenediamine tetraacetic acid (EDTA) or urea. An attempt was made to verify the possibility that the inactive form of protease was present, and the influence of these substances on the activity of the protease in the fraction which sediments at 105,000*g* was therefore studied. The enzymatic activity was found to be reduced by EDTA at a concentration of 1×10^{-2} *M* by 70–80 per cent and the enzyme was completely inactivated by 4 *M* urea. The decrease in activity caused by EDTA was about the same in the fraction of ribosomes and in the cytoplasm and thus the presence of inactive protease in the ribosomes appears unlikely. The results obtained from fractions isolated from disrupted spheroplasts were checked by using material homogenized in the bacterial press[7]. It was found that in this case the enzyme was also distributed quite evenly between the fractions sedimenting at 10,000*g* and the cytoplasm. Here, too, only a small part of the whole amount of protease was found in the fraction of ribosomes.

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VIROLOGY

Presence in Horse Serum of Two Inhibitors of Avid *A*2 Influenza Virus Strains

Recently it has been shown[1-3] that normal sera from some animals contain substances which inhibit specifically the avid[4] *A*2 influenza virus strains. Attention has been paid to these substances, as they exhibit not only hæmagglutination-inhibiting activity, as the α-inhibitors, but also virus-neutralizing activity.

In our laboratory two inhibitors of avid *A*2 influenza virus strains, showing different mobilities in the electric field and differing also in other properties, were isolated from normal horse serum.

The faster-moving inhibitor, designated as glycoprotein inhibitor, was isolated from the lyophilized sulphosalicylic acid filtrate of the serum[5] by extraction with an equal volume of 90 per cent phenol at 65° C. for 30 min.[6]. The water layer was freed of phenol by dialysis and then lyophilized. The substance obtained showed a single boundary on electrophoresis and sedimentation in the analytical centrifuge. It was of a glycoprotein nature, containing 18–20 per cent hexoses, 14·8–16 per cent sialic acid, 11·5–12·5 per cent hexosamines and 38–40 per cent polypeptides. 1 mgm. of this glycoprotein contained 6,400–12,000 hæmagglutination-inhibiting units against 4 hæmagglutination units of the avid *A*2 (Singapore) 57 influenza virus strain (eighth egg passage) and neutralized 100–1,000 $TCID_{50}$ of this virus up to 1 : 1,024 dilution. The action of a 0·1 per cent solution of crystalline trypsin at *p*H 8·0 and 37° C. for three days lowered the inhibitory activity of the inhibitor 50–100 times. 400–800 units of receptor destroying enzyme have split 43–50 per cent of the total sialic acid from 2 mgm. of the glycoprotein, without affecting the activity of the inhibitor.

The slowly moving inhibitor, designated as γ-globulin inhibitor, was isolated from horse serum by precipitation with a 0 01 per cent solution of 'Neokal *B*' (commercial preparation of a butylnaphthalene-sulphonate) in phosphate buffer *p*H 6 4 and 0·02 ionic strength. The serum was mixed at 0° C. with the 'Neokal' solution in the ratio 1 : 30, the precipitate separated by centrifugation, redissolved in saline and dialysed against distilled water. During the dialysis the euglobulins, containing the bulk of the biological activity, were precipitated. They were used in this work without further purification. The electrophoretic and sedimentation patterns of this substance showed the presence of two components. 1 mgm. of the γ-globulin inhibitor contained 400–800 hæmagglutination-inhibiting and 512–1,024 virus-neutralizing units.

Both inhibitors isolated from horse serum were specific, in that they reacted only with the avid *A*2 influenza virus strains. Potassium periodate in 0·001–0·005 *M* concentrations completely inactivated the inhibitors at room temperature in 15 min.

The two inhibitors were separated from their mixture by paper electrophoresis using diethyl-barbiturate buffer *p*H 8·6 as electrolyte and recovered by elution of 1-cm. strips. The maxima of the hæmagglutination-inhibiting activity coincided with those of proteins, this being in agreement with the results obtained by other authors[7] with unfractionated horse serum.

Table 1. Hæmagglutination-Inhibiting Activity of the Glycoprotein and γ-Globulin Inhibitors after Different Treatments

Treatment, fraction, original concentration	Hæmagglutination-inhibiting units per ml.	
	Glycoprotein inhibitor	γ-Globulin inhibitor
'Neokal', supernatant, 2 mgm./ml.	32,768	0
'Neokal', precipitate, 35 mgm./ml.	no precipitate	16,384
Sulphosalicylic acid, filtrate, 20 mgm./ml.	131,072	0
Sulphosalicylic acid, precipitate, 35 mgm /ml.	no precipitate	2,048
Heating at 100° C. for 30 min., supernatant, 5 mgm./ml.	131,072	64
Heating at 100° C. for 30 min., precipitate, 5 mgm./ml.	no precipitate	0

Table 2 Some Properties of the Horse Serum Inhibitors against Avid *A*2 Influenza Virus Strains

Properties	Glycoprotein inhibitor	γ-Globulin inhibitor
Sedimentation constant (S_{20})	3 66 *S*	14 7 *S** and ~ 5·9 *S*
Mobility in the electric field at *p*H 8·6	with α-globulins	between β- and γ-globulins
Solubility in distilled water	soluble	insoluble
Hæmagglutination-inhibiting units per mgm.	13,107	409–1,638
Virus-neutralizing units per mgm.	1,024	512–1,024

* Main component

The differences in some properties of the inhibitors (solubility, sedimentation-rate, mobility in the electric field, hæmagglutination-inhibiting and virus-neutralizing activities) as well as the changes in their hæmagglutination-inhibiting activity after heating at 100° C. and treatment with 'Neokal', sulpho-salicylic acid and phenol are presented in Tables 1 and 2.

On the basis of the results so far obtained we may conclude that normal horse serum contains at least two specific inhibitors of avid *A*2 influenza virus strains. The inhibitor moving faster in the electric field is an acid serum glycoprotein, and the slow one—so far not characterized in detail—is of a γ-globulin nature.

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SOIL SCIENCE

Soluble Aluminium in Plants and Soils

Highly soluble aluminium concentrations in extracts of certain acid soils were first noted at least as early as 1904 [1]. It has been commonly explained as being due to aluminium ions held by chemical and physical means within the mineral part of the soil[2]. This mechanism usually requires a close correlation between soil pH and the concentration of soluble aluminium[3]; but, such correlation does not always occur.

The idea of accumulation of aluminium by plants is very old[4]. 'Aluminium accumulators', defined as plants which habitually accumulate appreciable quantities of aluminium in their tissues, have been regarded as a source of aluminium in the soil[2]. An exhaustive study of the aluminium content of leaves of plants in herbaria and from the British Colonies was made by Chernery[5,6]. Without considering the aluminium content of soils, he assumed that those specimens with more than 1,000 p.p.m. soluble aluminium according to the aluminon test would be accumulating species and those with less present in the leaves would be normal species. Various authors[7,8] have since used these data to explain soluble aluminium accumulation in soils, while Lovering[9] has suggested that it is a possible origin of bauxites and laterites.

Certain species are also thought to be dependent on a sufficiently high content of soluble aluminium in the soil for normal healthy growth[2]. Simonson[10] has suggested that there should therefore be indicator species to help in soil survey work. As Hutchinson[2] points out, there are undoubtedly many chemical forms in which soluble aluminium can occur in soils, and it can probably exist in appreciable quantities at all levels of pH.

During a detailed soil survey of an area of 30,000 acres on the coastal plain of British Guiana, a special study was made of the occurrence of species in the natural vegetation cover in relation to the distribution of soluble aluminium in the soil. The soluble aluminium could not have come in from outside, while drainage conditions and past history precluded the objections put forward by Chernery[11] in reply to Polynov[7]. Both high and low concentrations occurred at field pH values in the range < 4–8·5. No correlation with plant species was obtained, but there was a marked direct relationship between the return and accumulation of organic matter in the soil and the amount of soluble aluminium present in the same layer. Where secondary bush was allowed to grow, higher figures were obtained for both the organic matter content and the soluble aluminium in adjoining areas which had had a similar land-use history up to 1936.

The outstanding thing about the climax flora and distribution of soluble aluminium content was the wide range of tolerance of all species (Table 1). In an adjoining area[12], concentrations of soluble aluminium up to 13,986 p.p m. alumina have been reported in the surface A_1 horizon with a similar but rather stunted vegetation cover. Clearly these species grow equally well under conditions of both high- and low-soluble aluminium content in the soil. This might be expected since the flora has entered the area with high concentrations from the adjacent interior[13], where the soils have very low values for soluble aluminium.

The only explanation of these results would appear to be that the plants are absorbing aluminium from the soil, accumulating it and returning it with the soil organic matter. Owing to the action of earthworms (which live at pH values at least as low as 3·5), and other microfauna which mix up the organic matter with the upper mineral horizons at the drier sites, and the generally swampy conditions, there is an enrichment of the surface layers of the soil in organic matter and in soluble aluminium (probably as a chelate). In the absence of signs of any other processes, levels of soluble aluminium as high as 13,986 p.p.m. aluminium can only be obtained in this way.

Table 1. TOLERANCES OF SOLUBLE ALUMINIUM CONCENTRATIONS IN THE ROOT ZONE OF THE MAIN SPECIES IN THE CLIMAX FLORAS OF THE COASTLANDS OF BRITISH GUIANA

Climax (ref. 16)	Species	Family	Soluble aluminium content of the root zone (Al^{3+} p.p.m.)
Palm marsh forest	*Adiantium intermedium*	Polypodiaceae	120–570
	Cecropia angulata	Urticaceae	6–713
	Desmoncus sp.	Palmae	41–645
	Euterpe edulis	Palmae	63–2,962
	Miconia racemosa	Melastomaceae	63–645
	Montrichardia arborescens	Araceae	16–2,962
	Pentaclethra macroloba	Leguminosae	63–962
Swamp forest	*Mauritia flexuosa*	Palmae	120–2,962
	Mikania micrantha var. *congesta*	Compositae	120–760
	Pterocarpus officinalis	Leguminosae	179–1,200
	Tabebuia insignis var. *monophylla*	Bignoniaceae	160–2,962
Herbaceous swamp	*Blechnum serrulatum*	Polypodiaceae	91–2,962
	Cyperus luzulae	Cyperaceae	37–1,200
	Eleocharos interstincta	Cyperaceae	14–2,926
	Jussieua nervosa	Oenotheraceae	312–2,926
	Leersia hexandra	Gramineae	16–2,962

The process could operate faster if a 'compound interest' effect operated. The whole idea of using artificial fertilizers is based on a similar principle. Even in leached tropical soils, accumulations from plant tissues cause the surface layer generally to be richer in plant nutrients than the deeper horizons. It would therefore seem not unreasonable to expect that the accumulation of soluble aluminium in leaves of plants would increase as the available supply of soluble aluminium in the soil is increased. There is some evidence for this. Church[14] and Stoklasa[15] found widely different values for the aluminium content of *Aspidium filix-mas*. Chernery[5] also reported two greatly differing amounts in the tissues of *Eleocharis equisitina* from different environments. Further work is now being carried out to verify those relationships.

Thanks are due to the Director of Agriculture for permission to publish this communication.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 13

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. E. Gombrich : "Theory and Observation in the Work of Leonardo da Vinci".

Royal Institute of Chemistry (at the North-West Kent College of Technology, Miskin Road, Dartford, Kent), at 7.30 p.m.—Prof. E. D. Hughes, F.R.S. : "Reaction Mechanisms".

Tuesday, February 14

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Royal Society of Medicine, Psychiatry Section (at 1 Wimpole Street, London, W.1), at 5.15 p.m.—Prof. Eugene Popov : "Soviet Psychiatry".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m —Mr. J. Jessop : "Some Psycho-acoustic and Engineering Aspects of Hearing Aid Design".

Institution of the Rubber Industry, London Section (at the National College of Rubber Technology, Holloway Road, London, N.7), at 5.30 p.m.—Dr. W. D. Rae : "Technical Aspects of Rubber to Metal Bonding". 7 p.m.—Research Topics by College Research Personnel.

University of London (at Imperial College of Science and Technology, London, S W.7), at 5 30 p.m —Prof. J. S. Rowlinson : "The Impact of Chemical Physics on Technology" (Inaugural Lecture).

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m —Prof. R. A. McCance: "The Control of Acid-base Stability in the Newly Born".* (Tenth of fifteen lectures on "The Scientific Basis of Medicine", organized by the British Postgraduate Medical Federation)

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Technical Teacher Training" opened by Dr. F. T. Chapman.

Society of Chemical Industry, Corrosion Group (at 14 Belgrave Square, London, S.W 1), at 6 p.m.—Mr. W. D. Jarvis . "The Occurrence of Fire-side Corrosion in Modern Steam Generators".

Royal Institute of Chemistry (at Norwood Technical College, Knight's Hill, West Norwood, London, S.E.27), at 7 p.m.—Mr. T. McLachlan : "The Work of a Public Analyst".

Wednesday, February 15

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Discussion Meeting on "The Meteorological Implications of the Distribution of Radioactive Fall-out".

Royal Statistical Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. Christopher Scott : "Research on Mail Surveys".

Institution of Chemical Engineers (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. P. M. S. Blackett, F.R.S. : "Science and Technology and the Developing Countries" (Hinchley Memorial Lecture).

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. Rudd "A General Theory of Depreciation of Engineering Plant".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. S. L. Rowles · "Biochemistry of Enamel".* (Last of three lectures on "The Scientific Basis of Dentistry" organized by the British Postgraduate Medical Federation.)

Society of Environmental Engineers (at the Imperial College of Science and Technology, South Kensington, London, S.W.7), at 6 p.m —Meeting on "The Development and Testing of Small Electronic Components".

Society of Chemical Industry, London Section (joint meeting with the London Section of the Royal Institute of Chemistry, at University College, Gower Street, London, W.C.1), at 6.30 p.m.—Dr. J. S. Anderson, F.R.S. : "Some Recent Advances in Inorganic Chemistry".

Society for Analytical Chemistry, Biological Methods Group (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Mr. C. J. Threlfall : "The Use of Enzymes in Analysis".

Thursday, February 16

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. F. C. Webb . "Useful Micro-organisms".*

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. E. Amoore : "Arrest of Mitosis in Roots by Oxygen-lack or Cyanide" and "Dependence of Mitosis and Respiration in Roots Upon Oxygen Tension" ; Mr. A. G. Morton . "The Induction of Sporulation in Mould Fungi".

Institution of Mining and Metallurgy (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Scientific Papers.

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. E. R. Reifenberg : "Solution of the Plateau Problem for m-dimensional Surfaces of Varying Topological Type".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. G. P. Lewis : "The Role of Bradykinin in the Control of Local Blood Flow".* (Eleventh of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institution of Electrical Engineers (at the Central Hall, Westminster, London, S.W.1), at 6 p m.—Mr. L. J. Davies : "Transistors and All That" (Faraday Lecture).

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. G. E. Bessey : "An Investigation of the Properties of Mortars".

Plastics Institute (at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Prof. G. Gee, F.R.S. Swinburne Award Address.

Royal Society of Tropical Medicine and Hygiene (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Symposium on "Fungus Diseases". Contributors Prof. W. St. C. Symmers, Dr. Ian Murray and Dr. Mary Marples.

British Institute of Radiology (at 32 Welbeck Street, London, W.1), at 8 p.m.—Mr B. B. Milstein, Mr. D. A. W. Edwards, and Mr. F. R. Berridge : "The Mechanism of the Cardia".

Friday, February 17

Institute of Navigation (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Symposium on "The Admiralty Chart", introduced by the Hydrographer of the Navy.

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. W. B. Thompson : "A Comprehensive Introduction to Plasma Physics".

Royal Society of Medicine, Epidemiology and Preventive Medicine Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Discussion on "Study of Control Methods in Tuberculosis". Speakers . Dr. James Grant, Dr. E. L. Feinmann, and Dr. K. M. Martischnig.

Society of Chemical Industry, Fine Chemicals Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. T. A. Blanco White · "Patent Protection for Chemical Inventions".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—The Right Hon. The Lord Adrian of Cambridge, O.M., F.R.S. . "Dreaming".

Saturday, February 18

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p m.—Mr. E. Roc . "Journey to Jebel Al Akhdar (Arabia)".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned .

Assistant Lecturer (with a good honours degree in either botany or agricultural botany and a special interest in mycology or plant pathology) in the Department of Agricultural Botany—The Registrar, University College of Wales, Aberystwyth (February 18).

Senior Technician (with appropriate H.N.C. or C. and G. Final Certificate or equivalent) in the Physics Department, for servicing electronics equipment—The Registrar, Brighton Technical College, Richmond Terrace, Brighton, 7 (February 18).

Lecturer in Electric Traction in the Department of Electrical Engineering—Head of the Electrical Engineering Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7 (February 20).

Lecturer or Assistant Lecturer (with special qualifications in applied mathematics) ; and a Lecturer or Assistant Lecturer (with special qualifications in pure mathematics) in the Department of Mathematics of the Faculty of Science—The Registrar, The University, Manchester 13 (February 20).

Biochemist (honours graduate, with some research experience) to assist Dr. R. M. C. Dawson on isotopic studies on lipid metabolism in rumen micro-organisms and in the ruminant digestive tract—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge (February 23)

Senior Lecturer (with an honours degree in mathematics, and preferably a higher degree and some experience of research) in Applied Mathematics at the University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 25).

Assistant Lecturer (with a good honours degree and special interests in mineralogy) in Geology—The Registrar, King's College (University of London), Strand, London, W.C.2 (February 28).

Lecturer/Assistant Lecturer (preferably with experience in the field of gas discharge or solid state physics) in the Department of Physics—The Registrar, University College of North Staffordshire, Keele, Staffs. (February 28).

Lecturer (with a university degree in electrical engineering, subsequent experience in research, in teaching, or in industry, and a knowledge of the fundamentals of nuclear engineering) in Electrical Engineering at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (February 28).

Soil Survey Officer (male, with a first- or second-class honours degree in science) **for the Soil Survey of Scotland**—The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen (February 28).

Lecturer (with a special interest in geomorphology and/or economic geography) **in Geography**—The Secretary, The Queen's University, Belfast (March 1).

Senior Lecturer or **Lecturer in Physics** at the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 4).

Assistant Lecturer in Physical Chemistry—The Registrar, The University, Hull (March 6)

Lecturer and an **Assistant Lecturer** (men or women graduates) **in the Department of Zoology**—The Secretary to Council, Westfield College (University of London), Hampstead, London, N.W.3 (March 6).

Lecturer (with an honours degree in engineering or science with research and/or professional experience in one of the fields of radio communications, radio physics, or information theory) **in Electrical Engineering** at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 6).

Lecturer or **Assistant Lecturer** (with a special interest in plant physiology or biophysics) **in Botany**—The Registrar, University College, Singleton Park, Swansea (March 11).

Technical Officer (with a university degree in science or equivalent qualification and interested in the application and development of techniques for teaching and research in botany and/or biochemistry) **in the Department of Botany**, to be responsible for the general supervision and maintenance of the laboratories and the control and training of technical staff—The Registrar, University College, Singleton Park, Swansea (March 11).

Lecturer in Psychology at the University of Auckland, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, March 15).

Post-doctoral Research Fellow in Organic Chemistry for work which will involve a chemical study of the biosynthesis of pteridines, under the direction of Dr. H. C. S. Wood—The Secretary, The Royal College of Science and Technology, George Street, Glasgow, C.1 (March 15).

Professor of Pure Mathematics at Rhodes University, Grahamstown, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, March 20).

Senior Lecturer in Physics in the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 31).

Senior Lecturer in Medical Statistics in the Department of Public Health and Social Medicine—The Secretary, The University, Edinburgh (April 1).

Agricultural Chemist (with a good honours degree in agricultural chemistry or chemistry, and at least two years postgraduate research experience in agricultural chemistry) in Kenya, to take charge of research projects into soil and plant nutrition problems of coffee—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD. 63/7/050/C1.

Assistant Systematic Botanist (honours graduate in botany or agricultural botany)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

Editorial Assistant (with a university degree in science or technology, and preferably at least two years postgraduate experience in science or industry)—The Manager, *Nature* Office, Macmillan and Co., Ltd., St. Martin's Street, London, W.C.2.

Lecturer (preferably with a special interest in deformation and fracture, a knowledge of solid mechanics, and experience in some industrial field) **in Metallurgy in the Department of Materials**—The Recorder, College of Aeronautics, Cranfield, Bletchley, Bucks.

Lecturer (with geochemical qualifications and familiar with one or more of the following techniques: optical or X-ray spectrography, silicate or radioactive analysis) **in Pure Geochemistry**—Prof. D. Williams, Department of Geology Imperial College of Science and Technology, London, S.W.7.

Master to teach Physics to AS level and some Mathematics to O level—The Headmaster, Westminster School, 17 Dean's Yard, London, S.W.1.

Master to teach Physics to AS level; Chemistry to O level; and, if possible, some junior Biology and Mathematics—The Headmaster, Bloxham School, Banbury, Oxon.

Research Assistant to help with current research on an immunological project connected with rheumatoid arthritis—The Pathologist, Stoke Mandeville Hospital, Aylesbury, Bucks.

Senior Lecturer (qualified to direct research) **in Physics**—Clerk to the Governing Body, Northern Polytechnic, Holloway, London, N.7.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Handlist of Darwin Papers at the University Library, Cambridge. Pp. 72. (Cambridge: At the University Press, 1960. Published for the University Library.) 10*s*. [1111

Supplement to the Catalogue of Lewis's Medical, Scientific and Technical Lending Library, including a Classified Index of Subjects, with the names of those authors who have treated upon them—Supplement 1957–1959. Pp. 306. (London: H. K. Lewis and Co., Ltd., 1960.) 10*s*. 6*d*. net. (To subscribers to the Library, 5*s*. net.) [1411

Research Defence Society. Conquest Pamphlet No. 12: Animal Experiments in the Diagnosis of Human Disease: Outstanding questions posed and answered by the Research Defence Society. Pp. 8. (London: Research Defence Society, 1960.) 6*d*. [1411

Science Museum, London. Book Exhibitions No. 4: Historic Books on Mining and Kindred Subjects. Pp. 32. (London: Science Museum, or H.M. Stationery Office, 1960.) 1*s*. 6*d*. [1411

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 122: Poultry Mites, Lice and Fleas. Pp. 8. (Belfast: Ministry of Agriculture, 1960.) [1411

Agricultural Research Council. A Bibliography of Farm Buildings Research, 1945–1958. Part 4: Buildings for Cattle. Pp. 84. (London: Agricultural Research Council, 1960.) 4*s*. 6*d*. [1411

Bulletin of the British Museum (Natural History). Zoology. Vol. 7, No. 1: Hearing in Cetaceans Evolution of the Accessory Air Sacs and the Structure and Function of the Outer and Middle Ear in Recent Cetaceans. By F. C. Fraser and P. E. Purves. Pp. 1–140+plates 1–53. 80*s*. Vol. 7, No. 2: Les Types d'Harpagophoridae de R.I. Pocock Conservés du British Museum (Natural History), (Myriapodes, Diplopodes). Par J. M. Demange. Pp. 141–179. 15*s*. (London: British Museum (Natural History), 1960.) [1411

Annual Report of the Medical Research Council of Ireland, 1959. Pp. 58. (Dublin: Medical Research Council of Ireland, 1960.) [1711

Other Countries

Indian Forest Bulletin No. 171 (5), (New Series), Entomology: A List of Insect Pests of Forest Plants in India and Adjacent Countries. (Arranged Alphabetically according to the Plant Genera and Species, for the use of Forest Officers.) Part 6: List of Insect Pests of Plant Genera 'G' to 'K'. By R. N. Mathur and Balwant Singh. Pp. 91. (Delhi: Manager of Publications, 1960.) Rs. 7.25 nP.; 11*s*. 6*d*. [1011

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles. Comptes Rendus de Recherches, No. 25 (Octobre, 1960): Travaux de Centre de Chimie Physique Agricole. Pp. 232. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1960.) 270 francs. [1011

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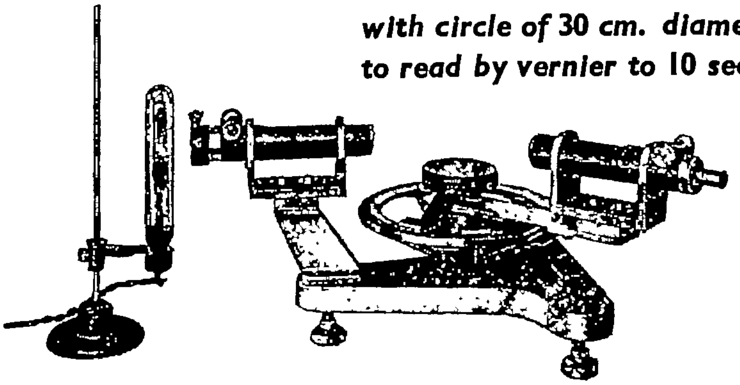

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UNIVERSITY ADMINISTRATION IN GREAT BRITAIN

IN its recent policy statement "Science and Education"*, the Association of Scientific Workers suggested that there is to-day a lack of contact between the universities and the industrial and government research organizations, and that co-ordination of the scientific effort of the universities with that of government and industry is only likely to be achieved by resolute and vigorous action. Looking essentially to a Royal Commission as the best method of achieving this, the Association suggests further that the time is ripe for a searching examination of the value of the traditional methods of science teaching and research in the universities in the context of the present and future needs of Great Britain. Such an inquiry, moreover, might also consider ways of ameliorating the present intensive competitive specialization in sixth forms of schools resulting from the existing scholarship system.

The Association is fully aware that university expansion should be considered in relation to the expansion of technical education also, both of the technical schools and colleges and of the colleges of technology; and, specifically, it recommends the elaboration of a national plan for the co-ordinated development of universities, technical colleges, teacher-training colleges, agricultural colleges, and colleges of art and music in Britain. This is as far as the statement goes; it does not go into the question of university government and administration. This aspect was raised at the Conference of Universities of the United Kingdom in London last December, when there was some discussion, and also disagreement, about the relations of teaching and research. There was firm support, however, for full participation of the academic staff in governing university institutions, and the dangers of external influence were stressed rather to the extent of over-shadowing the importance of securing local support and understanding and of the value of the independent advice and experience which could be drawn from outside the academic world, on which Sir Arthur Rucker rightly laid stress in his recent report.

Some re-assurance on university autonomy, particularly in respect of the new institutions, was offered to the Conference by Viscount Mackintosh of Halifax, but this question of university administration has been brought forward in recent issues of *Science and Freedom*†. The question is clearly one which lies well within the terms of reference of the Robbins Committee, and it is essential that some clear and constructive thought should be given before the pattern of university expansion and the interrelations of university institutions with other institutions of higher education are determined. Indeed, such thinking may well be the essential condition of the continuance of academic autonomy in an age in which university institutions are increasingly dependent upon public finance.

Some four years ago, in an article entitled "Self-Government in Modern British Universities", Sir Eric Ashby suggested that it is essential for academic freedom in a university that the academic staff should have *de facto* control of the admission and examination of students; the curricula for courses of study; the appointment and tenure of office of academic staff; and the allocation of income among the different categories of expenditure. The last-named, however, constitutes the real difficulty, and Sir Eric, who recognized freely the important part that laymen on university councils have taken in defending academic freedom, pointed out that, in speaking of the responsibility of the university council for the wider interests of the university in the community, he does not mean the interests of the community in the university. Furthermore, academic freedom depends less on the formal constitution than on the spirit in which it is interpreted in practice by the governing body, in collaboration with the academic staff.

These points, and the further one that the main direction of flow of new ideas and proposals, in contrast with the flow of finance, is from below upwards and not in the reverse direction, are to be remembered in considering the controversy that has been provoked by Dr. A. P. Rowe's broadcast and his paper in *Science and Freedom*, entitled "The Problem of Freedom and Efficiency in Universities", on which that broadcast was largely based. Equally they have reference to the discussion on the place of the independent research institution arising out of an earlier broadcast talk by Prof. A. H. Cottrell, entitled, "Scientists: Solo or Concerted", which was published in *The Listener* of September 15.

Particularly in his broadcast talk, Dr. Rowe challenged the existing pattern of research and teaching in British universities, and the appropriateness of some of the arrangements made in the past for much smaller numbers of students, for the conditions of to-day when the numbers of students are not only much larger but also there are liable to be fewer students with first-class minds or from homes with a tradition of learning or culture. Supporting Baroness Wootton's suggestion in the House of Lords debate on May 11 that some inquiry should be made into the distribution of teaching and research among the staffs and faculties in different universities, he urged further that university research should be located primarily in institutes associated with universities and not in self-contained university departments. In addition, he suggested that more attention should be given both to the quality of university teaching and to the needs and treatment of the ordinary student.

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* Science and Education: a Policy Statement by the Association of Scientific Workers. Pp. 16. (London: Association of Scientific Workers, 1960.) 1*s*.

† Committee on Science and Freedom. Bulletin No. 16: Freedom and Efficiency: Problems of University Administration in Britain and the Commonwealth. Pp. 32. (Manchester: Committee on Science and Freedom, 818 Wilmslow Road, 1960.)

It is from a re-assessment of the tasks of universities in Britain along such lines that Dr. Rowe proceeded to discuss the administration of a university. He visualizes a university organized flexibly, with interchangeable staffs and common social, library and other facilities, to undertake the three tasks, first, of teaching a majority of earnest, intelligent but not brilliant students, mostly from homes with little tradition of learning; secondly, of dealing with more senior students, capable of standing on their own feet and benefiting from contact with great minds, and who need advanced courses and training in research methods; and thirdly, with research, calling for continuity of effort and team-work. To achieve these ends, Dr. Rowe calls for the elimination of departmentalism and for a real measure of authority above the professorial level. This he would place in the hands of a very few able and experienced academic men with few other duties and appointed for a long period.

There is considerable foundation and support for the strongly held belief in the interdependence of research and teaching, but it will be remembered that the balance between teaching and research was one of the questions which Lord Simon of Wythenshawe suggested for inquiry by a Royal Commission or special committee. Whether it will be examined by the Robbins Committee remains to be seen, but Dr. Rowe has the support of Lord Beveridge and others in suggesting that more attention needs to be given to teaching. Nothing but good could come from any steps taken to emphasize the teaching functions of the university and to raise the standard of teaching, either by the revision of courses, the introduction of new methods, or by supervision of untrained and inexperienced teachers.

Nevertheless, Dr. Rowe has pointed out a number of weaknesses, which require administrative action for the most part if they are to be corrected, whether or not a change in the administrative structure of the universities is involved. A system which ensured that teaching ability drew its recognition and reward, as well as distinction in research, would of itself assist in putting university teaching back into its proper place; but Dr. Rowe merely distinguishes between learning and teaching, and on this point the analysis should go deeper. In intellectual work, both in the communication of knowledge and in the discovery of new knowledge, it is quality that counts; and in the expansion of university education, too great care cannot be taken to safeguard the pursuit of excellence. The outstanding danger is that of mediocrity. Great thinkers and scholars bring inspiration and stimulus not merely to the little cluster of research workers immediately around them, but also to successive generations of students in the whole university.

What seems clear enough is that some investigation of the quality of the research work being done in university laboratories, particularly with reference to the resources available and in comparison with that being done in the research institutes, of both independent foundations and of the Department of Scientific and Industrial Research, might well be worth while. Similarly, it would seem that teaching methods and courses should be re-examined in the light of the increasing numbers of university students of moderate ability with little tradition of learning. The second inquiry might appropriately be made by the individual universities, and the problem appears to be well to the front in the thinking of those responsible for the organization of the new universities in Britain. The first, however, calls for wider and independent examination, such as might, for example, be appropriately initiated by the Minister for Science or his Advisory Council for Scientific Policy. It obviously lies rather outside the scope of the Robbins Committee, though inquiry might well lead to findings with important administrative implications—as indeed is foreshadowed in the last report of the Advisory Council on Scientific Policy. Moreover, Prof. Cottrell has pointed out that the problems of administration of the research institute are very similar, and the implications are important if waste of national resources is to be avoided.

Despite, however, the approval given by Sir Arthur Rucker in his recent report to certain aspects of university administration, there appears to be a *prima facie* case for an independent examination of the whole structure of university administration in the light of the needs of to-day. Apart from the internal stresses that arise inevitably out of the sheer numerical size of expanding universities, the problems of adjusting the claims of teaching and research, those of research between the different disciplines and fields of knowledge, and particularly the relation between available national resources and what is being done elsewhere, either in other universities or in government or independent research institutions, are growing in complexity. It is already clear that more attention will have to be given to university co-operation, and increasingly the demands of university administration call for the separation of policy and executive management. This is not to suggest that a solution is to be found by imparting unmodified the type of organization which characterizes large, efficient and progressive business organizations. It is perhaps worth noting that the Committee under the chairmanship of Sir David Hughes Parry which reported a year ago on the New Zealand Universities (see *Nature*, 187, 107; 1960), besides recommending the establishment of a university grants committee, suggested governing bodies for the new universities of not more than twenty, including eight lay members, four of high standing in the Dominions and nominated by the Governor-General, and four co-opted by the governing body itself from those interested in the work and progress of the university and likely to give weight and authority to the university in the community. While the governing body has the final decision, it has full powers of delegation; further, the rights of the professorial board to be consulted on and to discuss any matter relating to the university, as well as its duty to advise the governing body, are emphasized. The Ashby Commission on Post-School Certificate and Higher Education in Nigeria takes a very similar line; its chapter in university government recommends a national universities commission, similar to the University Grants Committee in Britain, and emphasizes the importance of both the academic representation and the lay element on the governing bodies of Nigerian universities, as well as insisting on the undisputed autonomy of these governing bodies.

The whole subject deserves some careful and perhaps independent study and hard and creative thought. Universities, and the nation, might well benefit if a few university administrators found their way to the Administrative Staff College, though little might result unless the organization of their universities enabled them to give effect to measures which would eliminate waste and result in more effective use of highly trained staff; initially such measures usually tend to involve additional expenditure on technicians or auxiliary staff or equipment, and such expenditure, even if comparatively trivial, is not always readily sanctioned under existing arrangements as the University Grants Committee well knows. Lord Simon of Wythenshawe, to whose original suggestion of a Royal Commission the appointment of the Robbins Committee was ultimately due, had such administrative matters clearly in his mind, though he referred more specifically to the need for some means of co-ordinating the work of universities and that of other institutions for higher education. Even if the Robbins Committee does not itself look at these problems of the government and administration of universities in some detail, its report should leave no room for doubt as to the importance of such a full inquiry, or where the responsibility for initiating, as a matter of urgency, such an investigation most appropriately rests.

EVOLUTION OF THE FLOWER

Die Evolution der Angiospermen

Von Prof. Dr. Armen Takhtajan. Pp. viii+334. (Jena: Gustav Fischer Verlag, 1959.) 44.90 D.M.

THIS is a comprehensive work in German dealing with the flowering plants as a whole as well as in detail. The author is a Russian—strictly speaking an Armenian—and yet can write lucidly in languages other than his own. I have had letters from him in quite good English, making one blush at one's feeble attempts to communicate in any other language than one's own.

The opening sections deal with matters applicable generally to phylogeny—such as progressive, adaptive evolution and specialization. Here he uses a term new to me—'heterobathmie'. It means that one set of organs may advance while others remain static or even degenerate. This in a way may be considered axiomatic, but is not always taken into account in discussing phylogenies.

Some stress is also laid on 'neoteny' (pp. 25 and 143)—a somewhat new term as applied to botany, though it has been in use for some time in zoology—meaning the possibility of evolution advancing from juvenile stages with the elimination of the adult ones. Takhtajan thinks neoteny might help in explaining the very compressed type of gametophyte to be found in the angiospermous embryo sac. He has elaborated the idea of neoteny in essays of a somewhat philosophical nature recently translated from Russian and published in English. Perhaps he is inclined to overstress neoteny, but to make any new view impressive and to invite criticism an overstatement is not without its advantages.

After the general section, the work proceeds to discuss individually the various parts of the angiospermous plant from the evolutionary point of view. Sections are devoted to stem, leaf, conducting systems, flower, inflorescence, pollination, anther, embryo sac, male and female gametophytes, seed, fruit and aril. He enters into great detail regarding the structure of the pollen grain and its value in phylogeny.

Though a believer in the woody angiosperm preceding the herbaceous, he has a section on the passage back of the herb to the shrub. He points out that this would seem to have occurred in the five families which he mentions (p. 38). In all such cases it is necessary to emphasize that the reversion should be revealed structurally, particularly in the vascular elements. Evolution does not revert backwards morphologically, but does at times physiologically, that is, functionally. Hutchinson's second edition of his *Families of Flowering Plants* had only just appeared and apparently was not in time for Prof. Takhtajan to comment on the former's rigid division of the Dicotyledons into two main lines of woody and herbaceous forms. These he keeps as separate evolutions from non-existing ancestors, and, now in his second edition, is bold enough to name the Lignosae and Herbaceae respectively. It would be going outside the limits of this review to comment further on this far-reaching division of the Dicotyledons, but it is a matter that the systematists will have to take seriously into account.

The third part of the work is systematic, taking the orders in succession with descriptions of each, and appending to them the families they each include. The author further groups the orders into a higher category termed 'Überordnung'.

A few points in the scheme of classification, which is largely based on those of Bessey, Hallier and Hutchinson, may be mentioned. The genus *Paeonia* is now generally recognized to be worthy of a family to itself, Paeoniaceae. Hutchinson keeps it in the order Ranales; but Takhtajan widens the gap, as it were, placing it in the order Dilleniales. *Nelumbo*, the lotus lily, is removed from the Nymphaeales and given an order to itself, the Nelumbonales. One distinction from the other water lilies is shown in the structure of its pollen grain. The willows and poplars, the Salicaceae, always difficult to place, Takhtajan has put near the tamarisks and daphnes. Hutchinson still keeps them near the catkin families.

The author appends to the systematic descriptions a linear list of orders and families. On running through this list one is drawn up sharply with such unfamiliar names as Brassicaceae, Fabaceae, Apiaceae, Lamiaceae, Asteraceae, Poaceae, and Araceae in place of such time-honoured ones as Cruciferae, Papilionaceae, Umbelliferae, Labiatae, Compositae, Gramineae, and Palmae. One can see the reason for this. The old familiar names, except one, do not end in the customary '-aceae'. Without doing away with the old names entirely, could not each be extended by the interpolation of 'Ac', thus making them all end in '-aceae'? It is now generally admitted that the old Leguminosae is better split into three separate families. Hutchinson has the three now grouped under the order Leguminales, but Takhtajan under Fabiales. Would not the former be better, thus preserving the old name legume, so long associated with this group of plants?

It seems to me that some uniformity should be adopted regarding the above families. This might be settled at the next International Botanical Congress; and I would suggest that if a committee be formed for discussing nomenclature the horticulturist should be consulted as well as the systematist.

A word of appreciation must be said for the very full bibliography given. This alone makes the volume very desirable. There are also three quite useful indexes. In fact the text is so well documented that a work of this character in English would make a desirable advanced text-book for students.

The author is cautious in not putting forward any new speculations as to the solution of the still great problem, the origin of the Angiosperms. Reading between the lines and from correspondence, his inclination is towards the Magnolian–Bennittalean view as to the primitive features of the flower. Engler's main classification and the Caytonialian speculations are scarcely mentioned, looking as if they were now outmoded. J. PARKIN

HISTORY OF WESTERN IDEAS

The Western Intellectual Tradition from Leonardo to Hegel

By Dr. J. Bronowski and Dr. Bruce Mazlish. Pp. xviii + 522. (London: Hutchinson and Co. (Publishers), Ltd., 1960.) 30*s*. net.

THE authors set out to expound the history of Western thought from the late-fifteenth to the early-nineteenth centuries on three principles which, they claim, are fundamentally new. One is comprehensiveness—"we are interested in the whole spectrum of the mind"; the second is that ideas should be related to events; the third is that ideas should be studied through the men who first conceived them. The combination of these three principles, which they call triangulation, is intended to produce the "integration of intellectual history" and so make a coherent relationship between science and the humanities. They lay high claim to originality in so doing, and their preface is unusually full of self-congratulation for being pioneers in such a task.

The result, in view of these claims, is nevertheless conventional. They do not, fortunately, attempt to cover "the whole spectrum of the mind", for they say little about theology, moral philosophy, æsthetics, literature and drama, the fine arts, or indeed any realm of thought other than the conventional realms of political, social, economic and scientific thought. Their other two 'principles' are certainly not original, and they adopt the episodic chronological method already only too common in histories of political thought: some biographical facts, summaries of a man's ideas in relation to his life and times, some generalizations about his importance, above all for our own day. Their prime aim, to integrate scientific thought with social and political thought, cannot be achieved by merely including a chapter on the scientific revolution alongside those on Leonardo and Machiavelli, or one on the Royal Society along with those on Hobbes, Locke and Descartes. Nor have the historians of ideas, of whom they speak so contemptuously, been so unaware of the influence of a Galileo, a Newton, or a Watt. The remark of Sir Charles Snow which they quote with such warm approval can scarcely go on record as one of his more felicitous or accurate statements: "The steam engine helped to shape the modern world at least as much as Napoleon and Adam Smith, *but only rarely do the historians admit the fact*". The book ends, moreover, around 1830, just when the authors' self-imposed task would become really important but also extremely difficult.

Most of the time the historical categories of the book are entirely conventional—superficial generalities about "rising middle classes", or about scientific, industrial, political 'revolutions'. Its historical judgments are at times, indeed, out of date. Although footnote homage is duly paid to the late Sir Lewis Namier, the text (p. 366) still asserts that George III "was determined to impose an absolute rule on America as on England". When more original the authors are apt to be extravagant. The resistance to Charles I after 1630 is described as "the first major revolution in the world" (p. xvii). The assertion that "the experience of the French Revolution and of Napoleon, the philosophy of a Hegel, marks (*sic*) the transition from dynastic empires and tradition to nation states and charismatic authority" (p. xvi) leaves inexplicable the next hundred years of European history, which saw the creation of new dynastic empires in France and Germany and their cheerful survival in Austria-Hungary, Russia and Turkey.

Nor are the authors consistently true to their avowed method. How can they hope to explain the ideas of the late-seventeenth century, including their all-important scientific revolution, without giving much more systematic attention to events and conditions in the Netherlands, or a clearer picture of the English Civil War? They are shaky on economic history (even apart from allegations that historians neglect the steam-engine), and whether on enclosures in England or serfdom in Europe they commit vague and misleading assertions. They (or their indexer) confuses Robert Walpole with Horace: and to speak of Rousseau's "one-party view" is to misunderstand a vital part of his theory. On the Royal Society and the Lunar Society of Birmingham the authors have much to say that is interesting but scarcely original.

However, when all these substantial defects have been noted, the book has its uses and its value. It is, on the whole, a successful piece of intellectual regurgitation. It is written in a lively, easy style, confident if sometimes over-confident. It has excellent bibliographical notes and admirable brief summaries of the main historical controversies. It directs attention, at least, to the need to widen and deepen and experiment in the study of intellectual history. As a piece of popularization it is enterprising, efficient, and as good as most. It is not more than that.

D. THOMSON

A TEXT-BOOK OF SURFACE CHEMISTRY

Physical Chemistry of Surfaces

By Arthur W. Adamson. Pp. xiv+629. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 96*s*.

PROF. ADAMSON has written a very useful text-book of surface chemistry, well balanced, full, and as up to date as anyone is likely to achieve in these days of over-voluminous and often hasty publication. It covers most of the field, including chapters on capillarity, the thermodynamics of liquid-vapour interfaces, surface films on liquids, electrical aspects of surfaces, and long-range forces. Five chapters deal with solid surfaces, one being a brief introduction to chemisorption and catalysis; and in three more, various practical applications, including wetting and detergency, flotation of minerals, emulsions and foams, and friction and lubrication, are competently discussed.

Very sensibly, the author has made good use of several earlier books, and he has added, and usually succeeded in satisfactorily incorporating, a very large amount of later work. The book is written primarily for first-year graduate students in American universities, but it should also be valuable to senior undergraduates and to anyone desiring to learn the principles, and many of the details and applications, of the subject. Many, indeed, who have carried out considerable research on surfaces can learn much from this book, in those parts of surface chemistry in which they are not specialists.

The book is also remarkably clearly written, with numerous useful analogies: for example, the difficulty of defining the area of a surface is compared with the difficulty of deciding what should be taken as the coast-line of Maine. There are a number of problems for the reader to work out at the end of many of the chapters: some of these are ingenious, and admirably suited to test the student's understanding.

I have few criticisms: perhaps there are more mistakes in historical matters, assignment of priority in discovery, dates, and spelling of names, than might have been expected: but once detected, these can easily be rectified in future printings. In places, particularly in discussing heterogeneous catalysis, he follows the modern fashion of attaching names to theories and mechanisms, and these are not always those of the originators. Few people realize that it was Langmuir who, in 1922, published quite detailed and well-worked-out kinetics of catalytic oxidation, assuming reaction between adsorbed atoms of oxygen and molecules colliding from the gas phase: yet in this book, as unfortunately in much current literature, another name is given to this mechanism while Langmuir's name is attached to a mechanism which, so far as I am aware, he did not advocate. Too frequent attachment of names, according to current and sometimes local fashion rather than historical fact, renders a large majority of papers unreadable to anyone outside the immediate circle of specialists to which the author happens to belong, and should be avoided if possible in text-books.

Apart from these quite minor blemishes, Prof. Adamson has made a most valuable contribution to the literature of, and education in, surface chemistry, and deserves hearty congratulation. The same cannot be said of the publishers, who have fixed a price that may well put the book out of reach of large numbers who would profit by owning it.

N. K. ADAM

ADVANCES IN INORGANIC CHEMISTRY

Advances in Inorganic Chemistry and Radiochemistry

Vol. 2. Edited by H. J. Emeléus and A. G. Sharpe. Pp. viii+392. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1960.) 12 dollars.

THE titles of the reviews in this volume are as follows: "Stereochemistry of Ionic Solids", by J. D. Dunitz and L. E. Orgel: "Organometallic Compounds" by J. Eisch and H. Gilman; "Fluorine-containing Compounds of Sulphur" by G. H. Cady; "Amides and Imides of the Oxyacids of Sulphur" by M. Becke-Goehring; "Halides of the Actinide Elements" by J. J. Katz and I. Sheft; "Structures of Compounds Containing Chains of Sulphur Atoms" by O. Foss; "Chemical Reactivity of the Boron Hydrides and Related Compounds" by F. G. A. Stone; and "Mass Spectrometry in Nuclear Chemistry" by H. G. Thode, C. C. McMullen and K. Fritze.

From the long-term point of view, the most important articles are probably the first two, which attempt explanation as well as summary. The chapter by Dunitz and Orgel should be read by anyone professing a knowledge of structural inorganic chemistry. Three of the other chapters are devoted to various aspects of the chemistry of sulphur and reflect the current great interest in the chemistry of the elements of the second short row of the Periodic Table—although it is rather unfortunate that there is some overlap between the article on sulphur acid amides and a recent review on sulphur–nitrogen chemistry in *Progress in Inorganic Chemistry*. The chapter on mass spectrometry is devoted largely to the study and theory of fission yields. From the point of view of physical techniques this volume has two chapters devoted to results derived by X-ray crystallography. This should emphasize that the only absolute method of structural determination is by crystallography, and it is to be hoped that these reviews will stimulate further the interest of crystallographers in the structures of inorganic compounds.

Both in the standard of articles and of production, this volume is fully up to the high standard of the first of the series. The general state of health of inorganic chemistry is shown by its apparent ability to support both this series and the parallel series of *Progress in Inorganic Chemistry*. There is a risk that there will soon be almost as many chemists writing reviews as are carrying out original work, but at the moment *Advances in Inorganic Chemistry and Radiochemistry* is proving an extremely useful and informative series.

D. W. A. SHARP

GRAPHITE: AN IDEALLY IMPERFECT MATERIAL

Graphite and its Crystal Compounds

By Prof. A. R. Ubbelohde and F. A. Lewis. Pp. xii+217. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 35*s*. net.

GRAPHITE is perhaps the best example of the fact that the regular three-dimensional structure of solids, so clearly revealed by the early work of von Laue and the Braggs, is only a first approximation to the truth. A study of its physical and chemical properties, many of which have become of great technical importance, reveals the significance of imperfections in the solid and demonstrates how far we are from possessing any refined means of measuring crystal defects.

It is against this kind of background that Ubbelohde and Lewis have written their monograph *Graphite and Its Crystal Compounds*, which brings together the vast literature on this subject for the first time. The authors do full justice to the fact that it is a wide range of materials which they are describing and not a single well-defined substance. During the past twenty years the number of technical uses and demands for graphite have greatly multiplied: for example, nuclear reactors have called for graphites with closely specified properties, such as purity, high-density and non-permeability. These needs have stimulated research by every kind of modern technique, but, as the authors say, our knowledge is still very uneven and imperfect. Their book provides a basic text as a starting point for the many physicists, chemists, engineers and others who seek to understand some aspect of graphite in relation to the wider, still imperfect, picture.

Rather more than half the book deals with graphite itself, in four chapters. The first of these is written from a crystallographic point of view, and sets out our present knowledge of 'ideal' graphite and the physical and chemical departures from this perfect model which make up the wide range of materials which are known and used. Emphasis is placed on the conception of graphite as the extreme case of the large aromatic molecule. Succeeding chapters deal with the more mechanical physical properties such as compressibility, elastic moduli and frictional behaviour and with the thermal properties and the thermodynamics of the graphite-diamond transformation. The latter discussion is followed by an account of related substances such as silicon, silicon carbide, boron nitride and borazon. Chapter 4, the longest in the book, collects together the large amount of data on electronic properties, derived from measurements of magnetic susceptibility, electrical conduction, thermoelectric power, Hall effect and optical properties.

The second half of the book deals with the chemical behaviour of graphite, as represented by the production at ordinary temperatures of the characteristic lamellar compounds (in which the carbon layers retain their identity largely unchanged) and by the conversion to volatile products at higher temperatures, sufficient to break up the bond structure, by oxidation or reaction with carbon dioxide, hydrogen or water. The lamellar compounds are classified broadly in terms of their bond structure and the electronic character of the predominating carbon macro-molecules. An account of their magnetic and electrical properties concentrates on showing how the general changes on compound formation, such as reduction of electrical resistance and of diamagnetic susceptibility, contribute to the general understanding of electron bands in graphite itself. A separate chapter is devoted to the still controversial structure of graphite oxide in which the carbon networks have probably become buckled. In the final chapter, on the complete chemical transformation of graphite, the emphasis is on the mechanism of the oxidation process, its dependence on surface and edge defects and crystalline perfection, with an eye, for example, on the development of oxidation-resistant graphites for refractories and nuclear reactors.

Throughout the book the discussion is extremely well furnished with literature references, and the list of these contains about a thousand entries, including many for 1959. The only obvious omission is the very recent work by electron microscopy which may have developed into a valuable contribution in time for a second edition, for this is assuredly not a subject where fact or interpretation will remain stationary for very long. G. E. BACON

ANIMAL BEHAVIOUR

Animal Behaviour

By Prof. J. L. Cloudsley-Thompson. Pp. xv+162+16 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1960.) 15*s*. net.

ALREADY it is traditional for books on animal behaviour to be illustrated by stylish photographs, and those accompanying Prof. Cloudsley-Thompson's text are exceptionally elegant. The text itself, unfortunately, is not of the same quality. The work is explicitly intended as a primer of animal behaviour for the non-specialist reader, and the diversity of the examples which are discussed makes for a certain freshness of approach; a discursive and imprecise presentation lessens their impact. Of nocturnal animals Prof. Thompson writes: "The carnivorous habit is less hazardous too at night, and competition is reduced when nocturnal carnivores such as beetles prey on sleeping diurnal forms such as butterflies". It is not only the layman who may be expected to find this type of generalization difficult to follow in the absence of any further exposition.

Many of the drawings appear to be merely decorative. For example, an account of phototonus in *Proctacanthus* on p. 9 is accompanied by a picture of two flies in bizarre attitudes, but the legend does not explain what has been done to them. Similarly, pp. 21–22 discuss neuro-muscular facilitation in the sea anemone, but the idealized kymograph tracings of the illustration bear no account of the method by which they were obtained. The number of different examples used to reveal the mechanisms of invertebrate behaviour precludes the adequate discussion of any one of them, and we are told little about the means by which zoologists have chosen to investigate them. This is a pity, for while controversy among behaviour workers may be highly technical, their experimental methods are often extremely simple, and of just the kind to appeal to the interested layman. The last chapter is headed "Displacement Activity and Human Behaviour", and is as superficial as its title would suggest. A. D. BLEST

Mambu

A Melanesian Millennium. By Dr. Kenelm Burridge. Pp. xxiii+296+17 plates (London: Methuen and Co., Ltd., 1960.) 42*s.* net.

MAMBU, pidgin English for bamboo, is in this work a name of a prophet of a millenium in which the spirits of the dead will bring the tools and consumers' goods of a developed culture for free distribution among their descendants of the Territory of Papua and New Guinea, who were recently of modern neolithic culture. The millenarian cargo-cult described began in the Territory more than sixty years ago, and has been previously discussed by several authors, including Haddon and Chinnery, Lawrence, Mead, Williams, Hanneman and Worsley. The cult has been viewed with some distaste by missionaries such as Hanneman, who notes that it is materialistic, and its prophets have, on occasion, been arrested by the administration, as they sometimes encouraged people to take no care for their self-subsistence, to slaughter their pigs and to build large and very quickly disused houses intended for the reception of the revenants with the tools and goods. Moreover, the prophets have sometimes preached that the secret of wealth was magical and was withheld from Papuans by missionaries, administrators, traders and settlers. There has not been a grave threat in the cult. Indeed, Hanneman noted with some disapproval of it that peoples of other countries organized revolutions, elections, changes of government, strikes and the writing of books on capitalism, democracy and utopia with aims of improving living conditions. The general idea in the discouragement of cargo-cult prophets has probably been to gain time until greater facilities for technical education are made available in the Territory, and until there are more Papuan students with an Australian education returned to it.

Dr. Kenelm Burridge describes incidents of the cargo cult in the Madang district and on the Island of Manam in good literary style and with a sense of the humour of situations. In particular, he discusses myths and dreams with appreciation of them, and what he terms the triangle of administrators, missionaries and Papuans with a detailed account of their angles.

R. F. FORTUNE

Fiori delle Ande

Da Alfonso Vinci. Pp. 68 (70 illustrations). (Bari: Leonardo da Vinci Editrice, 1960.) 1,500 lire.

THIS is the first pocket flower-book, of the kind so well known to us in Europe, for the Andes and perhaps for the whole of South America. The area covered is the complex series of sierras and páramos above Mérida, in Venezuela, but many of the plants illustrated have wide distributions in the Andes. The excellent short introduction, in Italian, describing the physical features, climate, geology and vegetation zones of the region, is far above the head of the average tourist and is more suited to the highly educated traveller or trained naturalist. Following this are three coloured illustrations of the three zones, tierra caliente, templada and fría. Then come the small pictures, 66 in number and also in colour, of individual flowers. The presentation of these is not worthy of the introduction, and has obviously been put together with undue haste. Many of the plants are described as "specie non determinata" or are assigned to wildly wrong families, when the identification is obvious to any botanist familiar with this flora. Yet the author thanks two distinguished local botanists for their help. It is a pity that this attractive little forerunner has been spoiled by such haste or lack of care. Even in the introduction there is the statement that *Draba* and *Pringlea*, in Cruciferae, are represented on the Andes, in the Alps and on the remote island of Kerguelen! At the top of p. 11 the second and third lines have been transposed.

N. Y. SANDWITH

Fundamentals of Nutrition

By E. W. Crampton and L. E. Lloyd. (Series of Books in Agricultural Science.) Pp. xi+494. (San Francisco and London: W. H. Freeman and Company, 1959.) 50*s.*

IN this text-book, which is planned as an introductory course in human and animal nutrition, related disciplines are briefly discussed in an opening chapter. The first section is concerned with nutrients and composition of the animal and of foods. In five further sections the subject is developed with regard to metabolism of protein, fat and carbohydrate, the nature of vitamins and of mineral elements, experimental studies on nutrition and nutrient requirements for man and animals. The text is illustrated with simplified diagrammatic representations of metabolic pathways and other physiological processes. Reading lists are appended to every chapter to encourage the student to become aware of the expanding literature on the subject of nutrition. The book would be a useful adjunct to a course in physiology where general principles of nutrition must be taught. While it does not set out to be a complete treatise on nutrition it does provide a good background to a wider study of the subject and can be recommended as a useful introduction to a rapidly growing science.

A. M. COPPING

Socrates and the Animals

By Dr. Elena Quarelli. Translated from the Italian by Kathleen Speight. Pp. 160. (London: Hodder and Stoughton, Ltd., 1960.) 12*s.* 6*d.* net.

MANY people are sentimental where animals are concerned, and attribute to them qualities that they do not possess—even anthropomorphic. Others go to the opposite extreme and dismiss them as objects incapable of life or feeling. In this book Dr. Elena Quarelli investigates the problem whether animals possess immortal souls. She does this both philosophically and logically.

She seeks information from Ancient Greek thinkers such as Aristotle and Plato, from the Bible and official representatives of Christian teaching, and from contemporary zoologists and animal psychologists. Her own theories are backed by personal experience of animals, and her anecdotes make interesting and amusing reading.

It was impossible to limit her inquiry solely to the animal world; the problem had to be seen within the context of the universe as a whole. She studies animals in relation to the whole purpose and meaning of life, including human beings. The book therefore has a sincerely religious and deep-thinking basis.

The writer combines a sympathetic but unprejudiced approach to the problem. Her findings will delight many, and can offend none.

B. J. G. MAITLAND

THE POSSIBILITY OF THERAPEUTIC APPLICATIONS OF BEAMS OF NEGATIVE π-MESONS

By DR. P. H. FOWLER and DR. D. H. PERKINS
H. H. Wills Physics Laboratory, University of Bristol

FOLLOWING a recent lecture by Prof. J. Rotblat given in the H. H. Wills Physics Laboratory at Bristol, on the applications of radiations in medicine, we have been led to consider the possibility of using beams of negative π-mesons for the treatment of tumours. It is well known that a serious limitation to such treatment, when γ-rays are employed, is the heavy dosage delivered to the healthy tissue in the path of the γ-rays. A typical distribution of the variation of dosage with depth, using γ-rays from cobalt-60, is shown in Fig. 2. Even when the dosage to the healthy tissues is distributed by employing several fields, the resulting toxæmia is commonly the factor limiting the amount of radiation which can be delivered to the tumour. In this situation, it occurred to us that beams of π^--mesons would have, at least in principle, a number of decisive advantages.

From the point of view of its nuclear composition, biological tissue can be regarded as made up of hydrogen, carbon, nitrogen and oxygen. When a π^--meson is brought to rest in such a medium it may be captured by any one of these atoms. When captured by hydrogen, however, the resulting π^--mesic atom migrates a short distance in the tissue until it encounters a heavier nucleus to which the π^--meson is handed over. As a result, all the π^--mesons interact, before decaying, with one or another of the nuclei carbon-12, nitrogen-14 or oxygen-16. In such interactions, about 20 per cent of the total rest-energy of the π^--meson, that is $\sim$ 30 MeV., appears in the form of α-particles and protons, very nearly all of which have ranges less than 1 mm. in biological tissue. A further 40 MeV. is expended in breaking up the nucleus, and the remaining 70 MeV. is carried off as fast particles, mostly neutrons, many of which (60–70 per cent) will escape from the tissue without interacting. For our present purposes, it is a favourable feature that capture of π^--mesons by light elements, especially carbon-12 and oxygen-16, leads to an exceptionally large release of energy in the form of short-range α-particles as compared with nuclear capture by other elements.

The range of a π^--meson of 52 MeV. is about 10 cm. in biological tissue, the maximum depth at which it is commonly necessary to irradiate tumours in the human body. If such a beam of particles is homogeneous in energy, there will be a straggling in range of about 2·5 mm. (see Appendix). Further, the scattering in the direction of motion of the particles as they pass through tissue, due to Coulomb interactions in distant collisions with nuclei, results in a lateral dispersion of their points of arrest relative to their original directions of motion.

The root-mean-square value of this lateral deviation is about 3 per cent of the mean range (see Appendix). For a range of 10 cm. therefore, most of

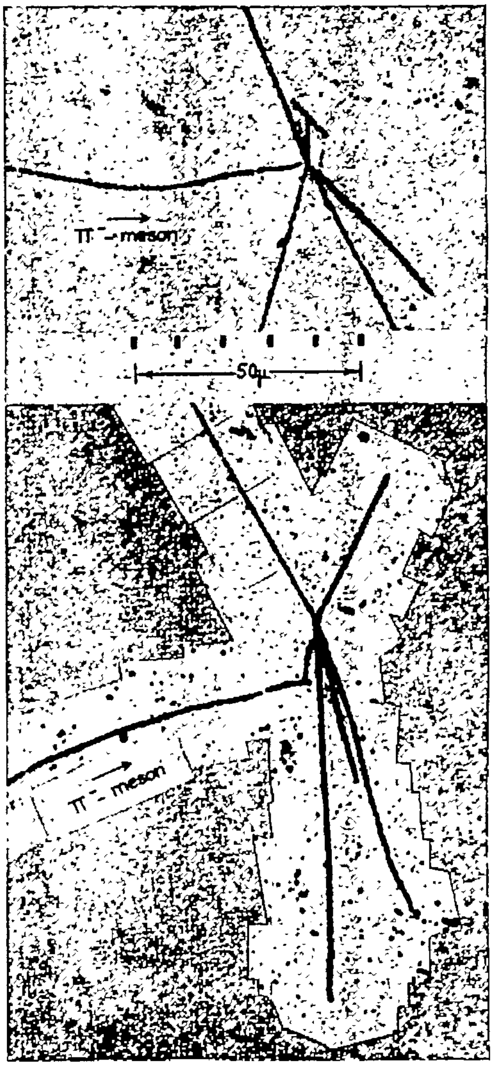

Fig. 1. Capture of negative π^--mesons: two photomicrographs of the nuclear capture of π^--mesons at rest in a nuclear emulsion. It is probable that each meson was captured by an oxygen nucleus of the gelatine. In the top example, one of the five charged secondaries is a nucleus of ^{8}Li. This nucleus undergoes β^--decay after being brought to rest, and produces two α-particles each of ≈ 2 MeV. that are emitted in opposite directions. The track of the β^--particle is not shown, as it dipped steeply with respect to the plane of the emulsion

In the lower example, it is probable that the charged secondaries were 2 α-particles and 3 singly-charged particles

In sensitive emulsions such as these, discrimination between tracks due to singly- and multiply-charged secondaries of short range is poor. In emulsions of lower sensitivity, the tracks of the singly-charged secondaries with range greater than ≈ 20μ can be picked out with confidence. The scale in microns gives the range of the secondaries in nuclear emulsion. Ranges in tissue would be about three times greater

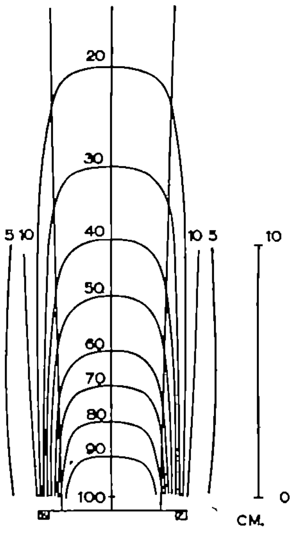

Fig. 2. Radiation field in biological tissue due to a collimated γ-ray beam from a cobalt-60 source

the points of arrest are distributed in a target area about 2 $cm.^3$ normal to the axis of the beam. The essential point emerges that the effective volume irradiated can be made less than the minimum volume of a tumour that it is usually considered desirable to treat.

In order to display the advantages of such a beam of π^--mesons, we show in Fig. 3 a calculated radiation field due to an initially parallel beam of these particles of energy 52 MeV. (range 10 cm. in tissue); the results may be compared with the computed field due to γ-rays in Fig. 2. The field produced by a proton beam of the same range is included. In each case, the beam at entry has a circular cross-section of area 2·2 $cm.^2$. In making the calculations, we have taken into account: (i) the change in loss of ionization of the beam as a function of depth; (ii) the effect of charged and neutral particles produced in the nuclear interactions of π^--mesons at the end of their range; (iii) the effect of the nuclear interactions in flight of the beam particles; and (iv) the effects of scattering and straggling on the dispersion of the beams.

In each case, no account was taken of the presence of other particles in the beam. In practice, a π-meson beam will contain an appreciable contamination of μ-mesons, electrons and neutrons, but we consider their effects could be made small.

In Fig. 3, the contours refer to the losses of ionization energy in rads (1 rad corresponds to an energy loss of 100 ergs/gm. in production of ion pairs). In order to obtain a numerical estimate of the distribution of the actual biological damage associated with this ionization, it is necessary to assume values for the relative biological damage per unit loss of energy, for the various radiations. This factor of relative biological efficiency is taken as unity for 220-keV. X-rays. It depends on the rate of loss of energy of the ionizing radiations, which are different in different regions of the radiation field. Along the path of the beams, the ionization is produced mainly by lightly ionizing particles, for which the relative biological efficiency will certainly be close to unity. In the region where the π-mesons are brought to rest, the ionization is mostly produced by heavily ionizing particles (mainly α-particles), for which the average value of the relative biological efficiency may be much higher, possibly about 6. At appreciable distances from the axis of the beam, the ionization is due to collisions of neutrons, for which the value of the relative biological efficiency is likely to be between 1 and 3. The details of the distribution of radiation damage will therefore depend on the sensitivity of the relative biological efficiency to the density of ionization of individual particles.

Unfortunately, there is at present no precise information on the values for relative biological efficiency of the different particles for the particular irradiation problem presented by the therapeutic treatment of tumours: a treatment which requires the destruction of practically all cells within a certain volume. For example, the U.S. National Bureau of Standards, in connexion with radiation protection[1], has recommended, for α-particles of energy a few MeV., a value of about 15. Snyder and Neufeld[2] have computed curves based on these recommendations for the radiation damage for various types of particles. On the other hand, direct measurements have been made by Storer *et al.*[3] for different

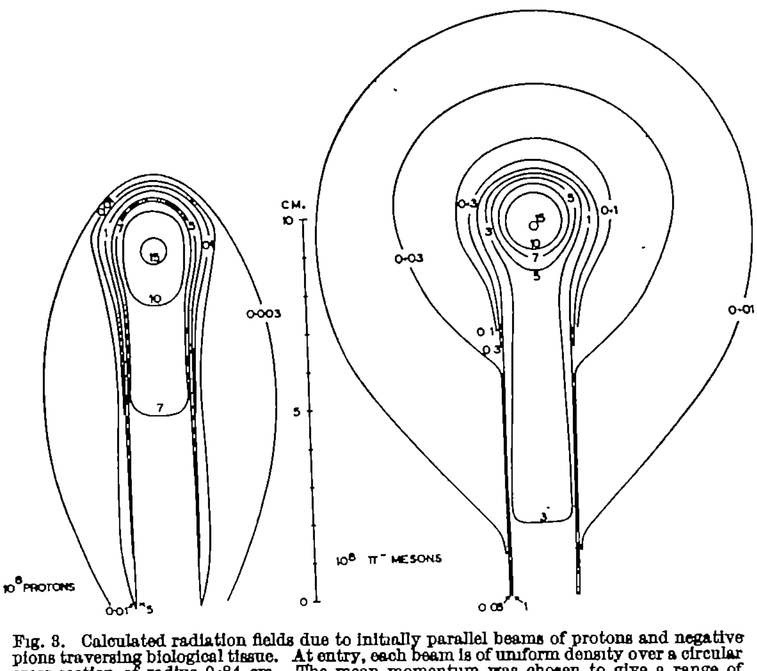

Fig. 3. Calculated radiation fields due to initially parallel beams of protons and negative pions traversing biological tissue. At entry, each beam is of uniform density over a circular cross-section of radius 0·84 cm. The mean momentum was chosen to give a range of 10 cm. in body tissue. The spread of momentum of each beam was taken to be rectangular in form and of total width given by $\Delta p/p = 4·5$ per cent; so that the most concentrated radiation damage occurs in a volume of roughly cylindrical form, of length equal to its diameter, centred at the end of the range (10 cm.)

In calculating the radiation intensity, allowance has been made for lateral Coulomb scattering of the beams and straggling in range. The total dose is made up of the effects of ionization loss due to (i) the incident beam and (ii) charged and neutral secondary particles from nuclear interactions of the protons in flight, and of the pions in flight and at rest. The numbers alongside the contours refer to the dose in rads for 10^8 incident pions, or protons

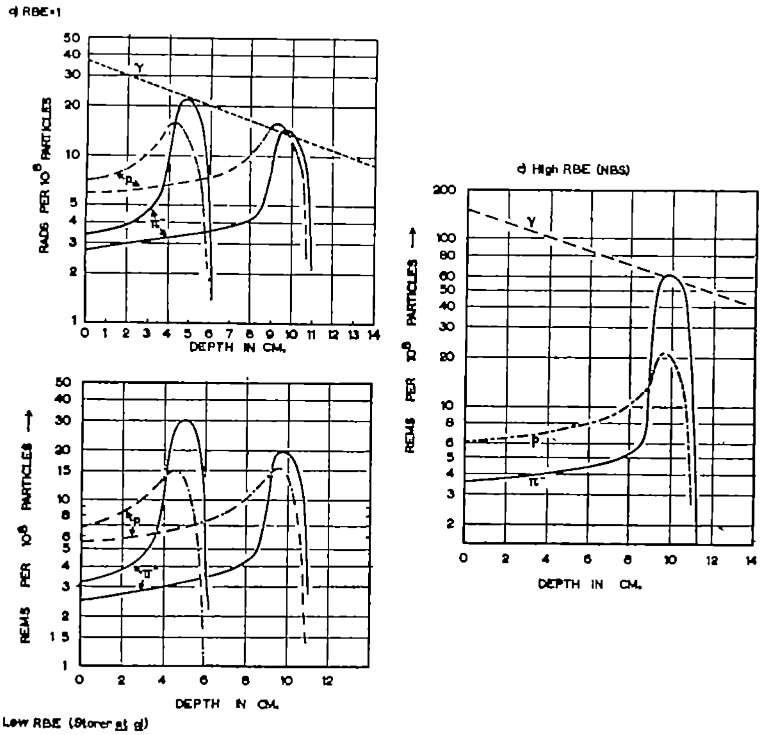

Fig. 4. (a) Axial radiation fields corresponding to the contours of Fig. 2. The (unnormalized) field due to a cobalt-60 γ-ray beam is also indicated
(b) and (c) Axial radiation fields of proton and pion beams of ranges 5 cm. and 10 cm. respectively, where the values for the relative biological effect of Storer *et al.* and the National Bureau of Standards recommendations are employed

particles producing lethal and other effects in rats and mice. For a variety of heavily ionizing particles, they obtain values of relative biological efficiency in the region 1·5–3·0. In view of these uncertainties, we have computed the distribution of radiation damage in rems (1 rem = 1 rad × relative biological efficiency) due to beams of negative pions, using in turn values of relative biological efficiency of unity for all particles, those of Storer *et al.*, and those recommended by the National Bureau of Standards. It seems probable that values appropriate to the present problem will lie between these extremes.

Fig. 4*a* represents the axial radiation dosage taken from the contours of Figs. 2 and 3. These fields correspond therefore to the use of a value of unity for relative biological efficiency. Fig. 4*b* shows the axial dose for π-mesons and protons assuming the values of Storer *et al.*[3], and Fig. 4*c*, that for the high values recommended by the National Bureau of Standards[1]. Calculations were made for ranges of both 5 cm. and 10 cm. The effect on the peak intensity of the lateral dispersion of the beam due to Coulomb scattering is very small for pions of 5-cm. range, and for protons of either range; but it reduces the peak intensity for pions of 10-cm. range by a factor of ~ 1·5.

The important feature illustrated by these results is that, whichever set of values for relative biological efficiency is taken, the dose received in a spherical volume centred near the end-point of the pion beams, and called here the 'tumour region', is much greater than that delivered in any of the surrounding tissue. The greatest dose outside the tumour region is of course in the path of the incoming beam, but the dose gradient even in this direction is very large.

The degree of concentration of the radiation damage in the region of the end-point is also illustrated in Table 1. These figures show the superiority of the pion irradiations over those by protons or γ-rays. The 'figures of merit' for the various beams have been specified in two ways: either by the ratio of the total dose delivered to the tumour to that in the whole of the surrounding tissue; or by the ratio of the dose per unit volume at the centre of the tumour to the maximum dose per c.c. delivered in a nearby region. For this purpose, we show the dose at the centre of irradiation divided by the maximum dose 1·5 cm. away from it.

The results show that, for single parallel beams of γ-rays and fast protons (and indeed neutrons), the radiation damage per unit volume in the tumour is no greater than that in the surrounding tissue, and that only a small fraction (less than 10 per cent) of the total body damage is confined to the tumour. Both these features are highly undesirable. In contrast, for a beam of π^--mesons, the damage per unit volume is many times greater in the tumour than in adjoining regions, and the total damage in the tumour region appreciably exceeds that in the whole of the surrounding body tissue. In principle, therefore, the advantages of a beam of π^--mesons appear to be very great.

The preceding results refer to irradiations over a tumour volume with linear dimensions of about 2 cm.; the lateral extent of the incoming beam, and the spread of momentum in the beams were so chosen as to irradiate such a volume nearly uniformly. If it were desired to treat a smaller volume, the characteristics of the beam could be altered accordingly.

In Fig. 5 we show the axial radiation field for pion and proton irradiations, with the fractional momentum spread of the beam, and its lateral extent, each reduced by a factor 2. This would correspond to irradiation of a tumour of one-eighth of the previous volume. The superiority of the π^--meson irradiation over the proton or γ-ray irradiation is apparent, and is somewhat more marked than when larger volumes are irradiated. The minimum volume within which one can restrict an appreciable fraction of the radiation damage is determined, for stopping particles, by the lateral spread produced by Coulomb scattering. As shown in the Appendix, this spread is nearly proportional to the depth of penetration (that is, the beam range).

Table 1

Radiation	(Tumour damage)/(External damage)		(Dose at tumour centre)/(Max. dose 1·5 cm. away)	
Tumour depth	5 cm.	10 cm.	5 cm.	10 cm.
Cobalt-60 γ-rays	0·11	0·07	0·9	0·9
Fast protons	0·08	0·08	1·0	1·0
Ending protons	0·9–1·3	0·4–0·6	1·4–1·9	1·4–1·9
π^--mesons	1·7–3·8	0·9–1·9	4·4–13	3·4–10

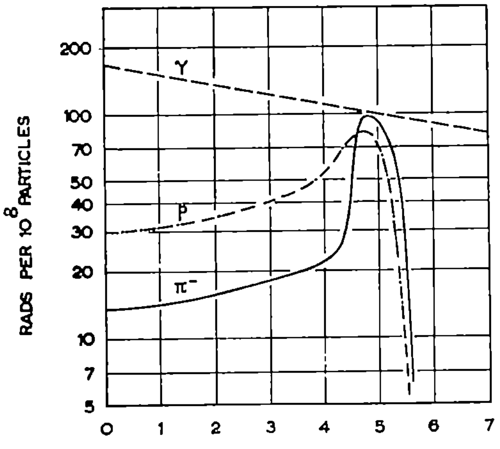

Fig. 5. Axial radiation field for the case of beams of radius 4 mm. and spread of momentum such that the particles end over a band of range 8 mm. in extent at a mean depth of 5 cm. Relative biological efficiency taken as unity for all particles

When employing γ-rays it is normal practice, in order to reduce the dose to healthy tissue adjacent to the tumour, to irradiate from several directions. The same advantage can be gained in the case of beams of low-energy protons or π^--mesons; for the latter, it may also prove practicable to produce a converging beam in a single exposure, because the momentum of the pions of the required range is quite low ($\approx$ 100 MeV./c.).

In view of these apparently great advantages, it appeared worth while to examine whether, with accelerators and their associated equipment at present available, which have been designed for the quite different purpose of making refined experiments with beams of fast elementary particles, it would be possible to secure irradiations of sufficient intensity in a reasonable time. For this purpose, on the basis of the results summarized in the figures, we estimate that a treatment of a tumour with an irradiation of 400 rems would require the arrest of up to 10^9 π^--mesons per c.c., or an incident beam of up to 5×10^8 pions/cm.^{2}, with a total spread of momentum of 4·5 per cent.

The Liverpool cyclotron, working with protons of energy 400 MeV. bombarding a thin beryllium target, can produce an analysed beam of π^--mesons of energy 90 MeV., and with a total spread of momentum of 4 per cent. The number of π^--mesons in the beam is $\sim 10^3$/cm.^{2}/sec. This beam is produced with a single quadrupole (focusing) magnet followed by a deflecting magnet for analysis of momentum. It is believed that a second stage of focusing would allow a large fraction of the original beam to be focused into a spot a few square centimetres in area. With the existing beam, the time of exposure required would therefore be up to 10^6 sec.

Doubtless improvements of intensity in a specially equipped machine could be secured by a suitable choice of the target element, by increasing the mean proton current and by employing focusing magnets of higher aperture, which would appear feasible for the low-momentum beams required. It seems likely also that the pion yield in the region of 50 MeV. could be made considerably bigger by a moderate increase in proton energy. In view of these factors, it appears not unreasonable to suggest that beams of π^--mesons 1,000 times more intense than those at present available in Liverpool would be technically feasible without any radical changes in present equipment, and that the shielding problem could be overcome. The corresponding exposure times for 10^9 particles would then be 1,000 sec.

These considerations make it desirable to determine in more detail the physical processes involved; and to make measurements of the actual distributions of the points of arrest of π^--mesons injected into biological tissue; on the distribution of the ionization due to both charged and neutral particles from the arrested π^--mesons; and of a number of other quantities. Such experiments are at present being designed and will be carried out in the near future. It will also be necessary to consider the question of the most advantageous forms of shielding from the high neutron fluxes generated at the target.

We may remark that our calculations indicate the marked superiority of beams of protons over γ-rays for therapeutic purposes. Although beams of protons are markedly inferior to pions, their production in sufficient intensity would be possible with technical methods already established, and they would have great advantages over γ-rays from cobalt-60.

Table 1 shows the ratio of the radiation dose delivered to a tumour compared with that in adjacent regions. The tumour is taken to be of radius 1·5 cm., and to be situated at a depth of 5 cm. or 10 cm. from the skin. In columns 2 and 3 the total external dose refers to that in a sphere of radius 10 cm. Calculations have been made for initially parallel beams of optimum width; and, for the low-energy protons and pions, with the optimum band of momentum. For columns 3 and 4 the figures apply to a single irradiation only. If irradiation is made from different directions, the values will increase by a factor which is practically the same for all four types of radiation.

The pair of figures given for the beams of slow protons and π^--mesons refer to a value of unity for the relative biological effect, and the high value recommended by the National Bureau of Standards, respectively. The fast-proton beam is assumed to be of sufficient energy to pass through the entire volume with only a small change in velocity.

Appendix

(*a*) *Lateral spread of a beam due to Coulomb scattering*. Consider a parallel pencil beam of initial energy E_0 and initial range R_0. Let $\overline{\alpha^2}$ be the mean square angle of multiple scattering of the particles in the beam per unit path-length when they possess residual range R. The total mean square lateral deviation of the points of arrest, $D_\perp{}^2$, is then given by:

$$D_\perp{}^2 = \int_0^{R_0} \overline{\alpha^2} R^2 \mathrm{d}R$$

$\overline{\alpha^2}$ for tissue is given by:

$$\overline{\alpha^2} = 10\cdot6/(p\beta)^2 \text{ (radians)}^2/\text{cm.}$$

where p is the momentum measured in MeV./c. and βc is the velocity of the particles at any point along their trajectory.

For the ranges R under consideration, $p\beta$ is given to sufficient accuracy for π-mesons by the relation:

$$p\beta = 24R^{0\cdot58} \text{ MeV./c.,}$$

where R is measured in cm.

Hence

$$\overline{D_{\perp}^{2}} = 1{\cdot}84 \times 10^{-6} \int_{0}^{R_0} R^{0{\cdot}84}\mathrm{d}R = 1{\cdot}0 \times 10^{-2} R_0^{1{\cdot}84}\ \text{cm.}^2$$

The root-mean-square projected value of $D_{\perp}$ along any direction perpendicular to the initial beam is $D_{\perp}/\sqrt{2}$ and is thus

$$D_{\perp\ \text{proj.}} = 7 \times 10^{-2} R_0^{0{\cdot}92}\ \text{cm.}$$

Thus for 52-MeV. π-mesons, for which $R_0 = 10$ cm., $D_{\perp\ \text{proj.}} = 0{\cdot}6$ cm. For other values of R_0, the ratio $(D_{\perp\ \text{proj}})/R_0$ remains almost constant. The lateral distribution of density of the points of arrest of the π-mesons at a distance D perpendicular to the beam is given by a Gaussian distribution:

$$\begin{aligned} I &= I_0 \exp - \frac{D^2}{2(D_{\perp\ \text{proj.}})^2} \\ &= I_0 \exp[-1{\cdot}4D^2],\ \text{for } R_0 = 10\ \text{cm.} \\ &= I_0 \exp[-5D^2],\ \text{for } R_0 = 5\ \text{cm.} \end{aligned}$$

At any intermediate point between entry and the point of arrest where the residual range is R cm., the root-mean-square lateral deviation $D_{\perp\ \text{proj}}(R)$ is given by:

$$D_{\perp\ \text{proj.}}(R) = D_{\perp\ \text{proj.}}\left[14{\cdot}9\left(\frac{R}{R_0}\right)^{1{\cdot}81} - 11{\cdot}5\left(\frac{R}{R_0}\right)^{2} - 4{\cdot}4\frac{R}{R_0} + 1\right]^{1/2}\ \text{cm.}$$

which may be approximated by:

$$\begin{aligned} D_{\perp\ \text{proj.}}(R) &= D_{\perp\ \text{proj.}}[1 - R/R_0]^2\ \text{cm.}, && R < R_0/2 \\ &= \frac{D_{\perp\ \text{proj.}}}{\sqrt{2}}[1 - R/R_0]^{3/2}\ \text{cm.}, && R > R_0/2 \end{aligned}$$

(*b*) *Longitudinal spread* (*straggling*) *of homogeneous beam*. The projections of the end-points of the pion tracks along the axis of the pencil are also spread out, due to statistical fluctuations in the energy loss. This distribution is again practically Gaussian. The root-mean-square value of this straggling about the mean range R_0 cm. in body tissue is:

$$D_{\parallel\text{RMS}} = 2{\cdot}6 \times 10^{-2} R_0^{0{\cdot}94}\ \text{cm.}$$

or $D_{\parallel}/R \sim 2{\cdot}3$ per cent for values of R_0 between 5 and 10 cm.

This figure for the straggling in range of a homogeneous beam of π^--mesons is probably smaller than one would require. So the longitudinal spread will be determined in practice by the spread in energy of the incoming π^--mesons; this can be adjusted by the movement of slits in the magnetic focusing system that would be employed. To obtain a spread of ± 5 mm. at 10 cm., an energy spread of ± 3 per cent in the incoming beam would be required. Such resolution is readily obtained.

We wish to thank Prof. C. F. Powell for his great interest and encouragement, and for discussions. We are also indebted to Mr. H. F. Freundlich for several discussions, and for Fig. 2 of this article. After preparing this report, we were informed by Dr. R. D. Hill, of the University of Illinois, that he had considered the problem previously (1958) and had circulated his conclusions in a private letter to certain medical authorities in the United States. These conclusions were somewhat different from those presented here, and the advantages of pion beams over existing beams of protons and γ-rays were not considered sufficiently substantial to warrant further pursuit of the matter.

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THE PERCEPTION OF SPEECH

THE growth of communication in the modern world has added a new dimension to applied psychology. In the air traffic control tower, the police information room, or the telephone switchboard of a large concern, men are daily subjected to a barrage of spoken messages with which they are expected to deal speedily and efficiently. In some of these situations, at least, human error can prove disastrous. It is therefore of obvious importance to ascertain the extent to which the human operator can handle a plurality of speech messages, and the factors which dispose him to error. What, in short, are the limits of the human operator conceived as a receiver and transmitter of spoken information?

This question was the theme of a Friday evening Discourse at the Royal Institution on November 18, delivered by Mr. D. E. Broadbent, director of the Medical Research Council's Applied Psychology Research Unit at Cambridge. Although taking the predicament of the air traffic controller as his point of departure, Mr. Broadbent was clearly more concerned with the theoretical upshot of recent work than with its contribution to immediate ends. He directed particular attention to the perception of simultaneous, or nearly simultaneous, messages, to which much research has been devoted during the past ten years. It is now clear that the familiar difficulty in 'hearing two things at once' cannot be ascribed primarily to masking or related interference at the physiological level, and seems due rather to difficulty in allocating the individual words heard to their respective contexts, that is, to perceptual confusion[1]. This difficulty, as Mr. Broadbent pointed out, may be appreciably reduced if the two messages differ in salient characteristics; for example, if spoken in different voices or appearing to originate from different points in space[2]. Indeed, spatial separation has been shown to be particularly effective in reducing error, more especially under conditions in which some of the information is irrelevant to the purposes of the listener[3]. Effective, too, is probability of message in the sense of the communications engineer. The extent to which different messages can be handled at the same time appears to depend not so much on the number of words reaching the two ears as upon the probability of each message. Indeed, two highly probable signals may in fact be perceived simultaneously. In general, Mr. Broadbent is on strong ground in advocating the view that the human brain, in virtue perhaps of its limited size, possesses a limited capacity for the processing of information.

A closely related problem is dichotic listening, that is, the perception of speech fed by independent channels to the two ears. Some years ago, Prof. Colin Cherry was able to show that the effects of feeding two different messages simultaneously, one to each ear, are entirely different from those that are obtained if the same messages are 'mixed' on a tape recording and the two ears stimulated identically[4]. In the case of the independent messages, the observer has no difficulty in listening to one or other message at will or in repeating it concurrently. He can, however, repeat virtually nothing of the 'rejected' message beyond perhaps the sex of the speaker. Yet this 'rejection' is not quite absolute: individual words arriving at the 'rejected' ear may sometimes be heard; for example, the listener's own name[5] or a word fitting more appropriately into the context of the message to which attention is being paid than that actually arriving at the 'accepted' ear[6]. There is evidence, moreover, of short-term 'storage' of the 'rejected' information. In earlier experiments, Mr. Broadbent demonstrated that if a short series of digit pairs was given dichotically, these digits could be correctly recalled, though not in their order of arrival. Almost invariably, the subject repeated first the digits fed to one ear and then those fed to the other[7]. More recent work has, however, suggested that this kind of selection is not governed exclusively by the ear to which attention is given. For example, if three monosyllabic words forming a short sentence are fed simultaneously with three digits such that words and digits alternate at the two ears, subjects are found to repeat first the sentence and then the number, or *vice versa*[8]. It seems possible, then, to listen for words as opposed to numbers just as one can listen to one ear as opposed to the other. At the same time, the existence of obvious differences between these two kinds of selection might suggest that they are governed by different types of cerebral mechanism.

Although few of the phenomena of speech perception find ready explanation in neurological terms, Mr. Broadbent argued in his discourse that the issues which they raise have an important bearing on auditory theory. For example, a vowel sound contains several different components at widely different frequencies, and to identify it requires that one should pick out the several components and treat them as pertaining to the same voice rather than the background noise. How does the auditory mechanism effect this discrimination? Although no very complete answer can be given, Mr. Broadbent suggested that amplitude modulation may play an important part. If a tuned system is subjected to amplitude modulation, the pitch of the resulting sound will, in appropriate conditions, correspond not to that of a steady wave having the frequency of the system but to that of the imposed pulses. In the case of the ear, one may argue that the pitch of a sound will depend not so much on the precise receptors stimulated (as traditional theory holds) as on some feature of the message travelling up the nerve fibres and affected by the rate of amplitude modulation. A likely candidate, Mr. Broadbent suggests, is the frequency of impulses in the auditory nerve. Although no direct neurophysiological evidence is available, Mr. Broadbent argues that experiments with 'synthetic speech' in which different parts of the spectrum can be modulated at different rates are well adapted to test this theory.

The study of speech perception and related problems is advancing with encouraging rapidity. Although the point of view of the applied psychologist influenced by communications theory is not always easy to reconcile with that of the sensory physiologist, the approach outlined by Mr. Broadbent has certainly served to throw valuable light on the limits of the auditory mechanism and on the selective processes which operate in the perception of speech. It may be hoped that the next step will be the translation of the models derived from information theory—which Mr. Broadbent has himself done much to develop—into those of neurophysiology. This possibility one recent writer at least regards as a great deal less remote than is commonly supposed[9].

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OBITUARY

Prof. H. T. S. Britton

PROF. HUBERT THOMAS STANLEY BRITTON died after a short illness on December 30; he is survived by his son and daughter. Born at Kingswood on April 22, 1892, he was educated in Bristol at St. George's Grammar School, the Merchant Venturers' College and the University of Bristol, graduating thence in 1914. He spent a short period as a grammar school master and then served during 1916–20 as chemist in the Aeronautics Inspection Department, and during 1920–25 as assistant lecturer at King's College, London. Between 1925 and 1927 he held a senior award of the Department of Scientific and Industrial Research at the Imperial College of Science and Technology, London, and then, after a year or so as lecturer at Norwood Technical College, he commenced his long association with the then University College of Exeter, first as lecturer and then, from 1935 until his retirement, as professor.

Despite a very heavy load of teaching—in the early years he was responsible for the whole of the honours teaching in physical and inorganic chemistry—Prof. Britton was extremely active in research, publishing numerous papers and directing the work of a considerable number of research students. Beyond the confines of his Department he was a prominent figure on the Senate, served at some time on the councils of the Chemical Society, the Royal

Institute of Chemistry and the Society of Chemical Industry, was for a period chairman of the board of governors of the North Devon Technical College, and was a popular extra-mural lecturer. He was instrumental in securing the establishment of the local sections of the Royal Institute of Chemistry and of the Society of Chemical Industry, and played a leading part in the preparations for the elevation of the College to university status.

Britton's research interests lay in the physico-chemical investigation of inorganic reactions in solution, mainly by measurements of the potentials of electrolytic cells incorporating hydrogen, glass and metal electrodes, but also including electrical conductance, diffusion and dialysis, phase-rule studies and polarimetry. He accumulated a mass of quantitative data relating to the precipitation of hydroxides, hydrated oxides and basic salts of numerous metals, and to the formation of complex ions involving a variety of acids and bases; he made notable contributions to the 'solution' chemistry of less-common elements such as vanadium, molybdenum and ruthenium, and with Robinson devised the well-known 'universal buffer mixture' which bears their names. Britton's *Hydrogen Ions*, which ran to four editions, is an indispensable reference book in every laboratory concerned with the physico-chemical behaviour of solutions of electrolytes.

Britton was the most approachable of men, ever warm-hearted and friendly, and was extremely popular with his students. He was a lively and entertaining lecturer whether to undergraduates or to an extra-mural audience in the heart of rural Devon, and he retained to the end something of the sceptic and the rebel. As head of department, he made himself freely accessible at all times to staff and students alike, and to colleagues from other departments. On his retirement in 1957 he was made emeritus professor, but his life in retirement was clouded by anxiety over his wife's failing health, and, in June 1959, her death.

Britton will live long in the affectionate memories of the many students who passed through his hands. His writings and researches contributed much to the chemistry of ionic solutions, and he laid the secure foundations on which the future development and expansion of the Chemistry Department at Exeter may confidently be based. S. J. GREGG

NEWS and VIEWS

The Space Vehicle 1961γ

A HEAVY Earth satellite was projected into orbit from a launching point in the U.S.S.R., probably at a time near midnight on February 11–12, and early on February 12 a space vehicle was launched from the heavy satellite, with the aid of a rocket which accelerated it to a speed greater than the escape speed of 11 km./sec. This space vehicle, which weighs 643·5 kgm. (1,419 lb.), was directed along a flight path designed to take it into the vicinity of the planet Venus, which it is expected to reach in the second half of May 1961, when the planet will be about 70 million km. from the Earth. At 9 hr. U.T. on February 12, the space vehicle was stated to be 126,300 km. from the Earth's surface at longitude 86° 40′ E. and latitude 6° 04′ N.; 24 hr. later, the distance had increased to 489,000 km., at longitude 88° 2′ E. and latitude 0° 53′ N. The vehicle's radio transmitter operates, on command, at a frequency of 922·8 Mc./s., and is to be switched on every 5 days. It is powered by chemical batteries, and these are re-charged by solar cells, which are orientated towards the Sun. The main purposes of the vehicle are to test the procedures for launching bodies into interplanetary orbits, to test guidance systems and communication at extreme distances, to investigate the size of the solar system, and to measure various physical properties of interplanetary space.

This vehicle, by far the heaviest yet launched into interplanetary space, represents a substantial step forward in space technology. Its flight was preceded by the launching of the heaviest Earth satellite, 1961β, weighing 6,483 kgm., which was put into a close orbit on February 4, and apparently served as a test vehicle for 1961γ. Any physical measurements made by the space vehicle in the neighbourhood of Venus will add to our scanty knowledge of this inscrutable planet; but it has not been disclosed whether the vehicle carries the terminal guidance which would be needed to make a very close approach to the planet.

The Science Museum Library: Mr. J. A. Chaldecott

MR. J. A. CHALDECOTT has been appointed keeper of the Science Museum Library in succession to the late Mr. H. T. Pledge. Mr. Chaldecott, who has been secretary to the Advisory Council of the Science Museum and deputy keeper in the Department of Physics since February 1957, was educated at Latymer Upper School, Hammersmith, and at Brentwood School. He read physics and mathematics at Borough Road College, graduating at the University of London in 1938. Early the following year he was commissioned in the Meteorological Branch of the Royal Air Force Voluntary Reserve; for much of the Second World War he was seconded to army formations, and his services earned him mention in dispatches. After the War he read history and philosophy of science at University College, London, obtaining his M.Sc. in 1949. He was lecturer in physics at Acton Technical College for a short period before entering the Science Museum, in 1949, as an assistant keeper. He catalogued the George III collection of scientific instruments, the heat and thermal instruments collection, and also edited the commemorative volume *The Science Museum: the First Hundred Years*. His published papers have been in the history of physics. He is a member of Council of the British Society for the History of Science.

Ministry of Aviation: Mr. F. H. Scrimshaw

MR. F. H. SCRIMSHAW has been promoted to the rank of deputy chief scientific officer. In 1939 Mr. Scrimshaw graduated in physics at the University of London with honours and was awarded the Granville research studentship as the best University of London physics graduate of the year. His first work at the Royal Aircraft Establishment, which he joined in 1939, was concerned with bomb-sights, followed by a spell during 1942–45 of research on airborne automatic dead-reckoning navigation equipment. On the formation of the Blind Landing Experimental Unit at Martlesham in 1945, Mr. Scrimshaw took charge of the flight control section which investigated such problems as the use of automatic pilots, leader cable guidance and automatic throttle control to aircraft landings. This work has come to fruition recently in the British Auto-land system which Britain is now actively sponsoring for use by civil airlines. In 1950 Mr. Scrimshaw returned to the Royal Aircraft Establishment, where he continued to work on inertia navigation systems, becoming an acknowledged expert in this field. He continued this work until 1958, when he became director of scientific research (electronics) at headquarters of the Ministry of Aviation. In this post Mr. Scrimshaw has been associated with long-term research projects in electronics in which the Ministry of Aviation has been engaged.

Anatomy at Cardiff: Prof. J. D. Lever

Dr. Jeffrey D. Lever has been appointed, in succession to the late Prof. J. S. Baxter, to the chair of anatomy in the University College of South Wales and Monmouthshire, Cardiff. Dr. Lever was educated at Westminster School and at Trinity College, Cambridge, which he joined as an Exhibitioner in 1941. He graduated in medicine in 1946, having pursued his clinical studies in St. Thomas's Hospital. After service in the Navy he joined the academic staff of the Anatomy Department in Cambridge as a University demonstrator. He was awarded the M.D. degree in 1954, and, in 1955, was appointed to a lectureship. During the academic year 1954–55 he was visiting instructor and research assistant in the Department of Anatomy, Washington University, St. Louis, where, under the direction of Dr. E. W. Dempsey, he received a training in electron microscopy. His interests as a research worker are concerned largely with the functional histology and fine structure of the endocrine glands. His publications include contributions on the adrenal gland, the islets of Langerhans, the parathyroids, and the carotid body. In 1956, on the recommendation of the Anatomical Society, he was awarded the Symington Prize, for original work in anatomy, by the Senate of The Queen's University of Belfast. He was elected to a fellowship of Trinity College, Cambridge, in 1957, and since then has been director of medical studies in the College. He is a member of the Council of the Anatomical Society.

A Co-operative Space Research Programme

Replying to a request in the House of Commons for a statement on progress achieved towards a Commonwealth and European space research programme, the Minister of Aviation, Mr. Peter Thorneycroft, said that at the Strasbourg Conference delegates had expressed keen interest in the political, scientific, economic and technological advantages of co-operation in the manufacture of launchers for heavy satellites. If an organization for this purpose was set up, it would be on a long-term basis, and could as its first programme use the British *Blue Streak* as a first stage, a French rocket as a second stage, and a third stage manufactured elsewhere in Europe. The United Kingdom and French Governments would now be asking their partners in the Conference to study its results with the view of deciding whether to form such an organization. The Federal Republic of West Germany was in principle to take part, but had neither the possibility of, nor the interest in, developing a rocket of its own for space purposes. As to co-operation with other countries, Mr. Thorneycroft said that Australia would undoubtedly be the place from which this programme, using *Blue Streak* as a first stage, would be launched, and we would, of course, welcome every co-operation with the United States. The British subscription would be on the basis of 33⅓ per cent which, on the estimated cost of £70 million, would be £23,300,000 over five years.

The Cotton, Silk and Man-made Fibres Research Association

In a written answer in the House of Commons on February 9, the Minister of Education, Sir David Eccles, as representing the Minister for Science, stated that it is proposed that the merger of the British Cotton Industry Research Association and the British Rayon Research Association into one association, to be called the Cotton, Silk and Man-made Fibres Research Association, will be effective from April 1, 1961. The annual expenditure will rise from about £490,000 to about £580,000 over the next five years, the industrial income being sought mainly from processors of cotton and man-made fibres by statutory levy under the Industrial Organization and Development Act, together with support from producers of man-made fibres. The new Research Association would occupy the present site of the Cotton Research Association at Didsbury, where the laboratories will be extended, and the laboratories of the Rayon Research Association at Heald Green will be sold. The research staff of the new Association will be drawn from the existing Associations, with adequate provision for redundancy, including retirement pensions, compensation and placing staff in other employment.

Committee on Higher Education in Britain

The Prime Minister announced, on December 20, that Lord Robbins would be chairman of the committee to consider higher education in Britain, and the terms of reference of the committee were announced (see *Nature*, February 11, p. 427).

The members of the committee have now been announced. They are: Sir David Anderson, formerly director of the Royal College of Science and Technology, Glasgow; Dame Kitty Anderson, headmistress, North London Collegiate School; Mr. A. Chenevix-Trench, headmaster, Bradfield College, Berkshire; Prof. J. M. Drever, professor of psychology and lately dean of the Faculty of Arts, Edinburgh; Mr. A. L. Elvin, director of the University of London Institute of Education; Miss Helen Gardner, professorial fellow, St. Hilda's College, Oxford; Sir Edward Herbert, an engineer, chairman of the governing body of Loughborough College of Technology and member of the Court of the University of Nottingham; Sir Patrick Linstead, rector of the Imperial College of Science and Technology, London; Sir Philip Morris, vice-chancellor of the University of Bristol; Mr. H. C. Shearman, vice-president of the Workers' Educational Association; Mr. R. B. Southall, general manager of the Llandarcy (BP) refinery, Swansea.

Research Expenditure on Fisheries

THE report of the committee of inquiry into the fishing industry, of which Lord Fleck was chairman, laid emphasis on adequate finance being available for scientific and industrial research bearing on the industry. In moving the second reading of the White Fish and Herring Industries Bill in the House of Commons on January 31, the Minister of Agriculture, Fisheries and Food, Mr. C. Soames, said that the Bill was not prompted by the Fleck report, to implement which would require major legislation and detailed consultation before such legislation could be drafted; and the importance of research for the fishing industry was stressed. Mr. J. H. Hoy suggested that if the normal fishing grounds were contracted, research directed towards the discovery of new fishing grounds should be undertaken; and Capt. M. Hewitson inquired specifically whether the possibility of fishing grounds off the coast of Ghana had been investigated. He thought the prospects of industrial fishing in that area were unlimited. Sir William Duthie also strongly urged adequate research to remove as much guesswork as possible from fishing, particularly as regards the incidence of shoal migration, and suggested that in this way we could find the answer to the depletion of the herring shoals in the North Sea. Other speakers agreed that, as the Fleck Committee pointed out, the fishing industry in many aspects lags behind other industries in the adoption of modern equipment, methods and organization; but it is also recognized that the question of research is closely linked with the economic aspects, since the application of its results increasingly involved large capital expenditure, beyond the resources of the small owner.

Mr. H. Hughes, who quoted the estimated figure of £1 million for expenditure by Britain on fisheries research in 1959–60, with a further £250,000 for research into handling and processing fish, referred also to the view of the Fleck Committee that a much more intensive research effort could profitably be directed towards exploiting more effectively the resources of the sea, and that to keep pace with changing conditions, the industry should devote a substantially greater sum to the development of improved catching and processing techniques. Questions raised in the debate relating to the closing of one of the three research stations at Aberdeen led the Secretary of State for Scotland, Mr. J. S. Maclay, to be at some pains to explain that the station which had been sold to Unilever, Ltd., which would continue research there, was the Ministry of Agriculture, Fisheries and Food Experimental Food Station, which was not primarily a fishing research station. The Marine Life Laboratory of the Department of Agriculture and Fisheries for Scotland, adjacent to the Department of Scientific and Industrial Research's research station at Torry, are both continuing and cover research into fish and the sea, biology, hydrography and the abundance and distribution of commercial fish stocks.

Telephone Services in Britain

IN opening a debate in the House of Lords on February 11 on the Post Office Telephone Service, prompted by the White Paper (Cmnd. 973) on *Post Office Capital Expenditure*, 1960–61, Lord Crook said that there are at least six main reasons why a steady figure for capital investment is essential for the telephone service. One of these is a steady research programme, and this is closely related both to the telecommunications industry and to the growth of electronics in the world of the telephone, where, Lord Crook believes, Britain is in danger of losing a great market. He questioned whether the Dollis Hill Experimental Establishment has been allowed to get ahead fast enough and given sufficient opportunity and finance. It is important, he said, to realize that the telephone service earns money, internally in industry within Britain, and also in respect of exports. The Earl of Listowel, supporting much that Lord Crook had said, added that the telephone service in Britain is backward because we have not put sufficient capital into the manufacture of telephone equipment and have not ensured for the Post Office the normal programme of capital development that any large business enterprise requires.

Replying for the Government on the debate, Lord St. Oswald assured the House that no limitation has been placed on research for electronic exchanges because of shortage of capital investment. It has been deliberately decided to employ different techniques in different functional sections of the experimental exchange at Highgate Wood, so that their merits can be compared from all points of view; the ultimate aim is an economic standard electronic exchange. Lord St. Oswald claimed that work is proceeding at high pressure to produce improved designs, so that British industry can retain its position in the forefront of telecommunication development and be able to offer in world markets electronic exchanges fully competitive with those of any other manufacturers. He pointed out, however, that development is not solely for export, and a besetting problem generally is the comparative lack of enthusiasm among telephone subscribers for anything new. The average residential telephone user makes less than one call a day on his instrument, and even putting business and residential subscribers together, the average is only 530 a year, compared with between 1,000 and 2,000 in Sweden, Canada and the United States. The Post Office, in conjunction with the University of London, has recently instituted a joint research programme into the sociology of telephone usage.

Research on Mental Hea'th in Britain

THE Minister of Health, Mr. E. Powell, in a written answer in the House of Commons on February 8, stated that after completing its full-scale review of mental health research early in 1959, the Medical Research Council established a new committee on clinical psychiatry, under Sir George Pickering as chairman, and one on the epidemiology of mental disorders, with Sir Aubrey Lewis as chairman. Each committee has appointed working parties to study special aspects of its field, such as the psychopathic personality, the value of drugs in psychiatry as assessed by clinical trials, and the use of national statistics of mental disorders. Of the total funds available to the Council for the past five years, the percentage spent on mental health research was as follows: 1956–57, 2·3; 1957–58, 1·8; 1958–59, 3·8; 1959–60, 4·4; 1960–61, 4·7.

Rostherne Mere, Cheshire, as a Nature Reserve

ROSTHERNE MERE and some surrounding woodland in north Cheshire have been presented to the Nature

Conservancy by the executors of the late Lord Egerton of Tatton, in accordance with his wishes. The area is to be declared a National Nature Reserve. Thousands of ducks which have learned to rely on the Mere as a sanctuary will thus continue to have the strict protection given during Lord Egerton's lifetime. The neighbouring Tatton Park, with its meres, having already been handed over to the National Trust and the Cheshire County Council, will provide for the recreational needs of the public. The area given to the Conservancy consists of 195 acres, and a nature reserve agreement with the new owner and the farmers of land immediately overlooking the Mere will cover an additional 183 acres. The hydro-biology of the area is of great interest and its supreme value as a winter resort for wildfowl, as well as a breeding-place for certain aquatic birds, is partly due to the strict control over access and disturbance maintained by the late Lord Egerton. In spring and autumn many migrant birds may be seen there resting on passage, and some rare species have been recorded. The late A. W. Boyd carried out numerous and detailed observations there for at least forty years and his work has made a great contribution to our knowledge of its bird fauna, which his friends hope to commemorate by building an observation post overlooking the Mere.

Peak District National Park

The third of the official guide books to Britain's National Parks has been prepared by the Peak Park Planning Board in association with the National Parks Commission, under the editorship of Patrick Monkhouse (*National Park Guides*, No. 3: Peak District. Pp. viii+90+26 plates. London: H.M. Stationery Office, 1960. 5*s*. net). Among the authoritative contributors is Lt.-Col. Gerald Haythornthwaite, who deals with the building traditions to be found in the Peak. Other contributions describe the geological structure and scenery of this National Park, its animal and plant life, and the marks made by man's occupation of the land since early times. There are also chapters on farming and industry in the Park to-day, as well as an account of the work done by the Peak Park Planning Board in preserving the beauty of the varied landscape and promoting its enjoyment. That the facilities of the Park are appreciated is shown by the way natives of grim industrial areas like South Lancashire, North Staffordshire and Sheffield seek solace in its open spaces. The guide book contains a gazetteer of places of interest which would be especially appreciated by visitors.

Changes in Sea-Level recorded in New Zealand

Hutton's doctrine that the present is the key to the past has been taken increasingly to heart by geologists in recent years. The sedimentary processes operating on the continental shelves and in various intertidal environments have received widespread attention since the War. Before older sedimentary rocks can be interpreted in terms of the Holocene, however, it is necessary to allow for any deviations from the norm which may have resulted from the Pleistocene glaciation. In particular, small changes in sea-level can influence sedimentation profoundly, and it is essential to determine any changes that have occurred as accurately as possible. Further evidence concerning world-wide fluctuations has recently been brought to light. In northern North Island, New Zealand, J. C. Schofield (*N.Z. J. Geol. and Geophys.*, **3**, 467; 1960) has been able to trace a number of elevated chenier ridges and to date the origin of several by radiocarbon determinations on shells. The area is believed to have been tectonically stable since the end of the Miocene period, so that the levels of the ridges may be interpreted in terms of changes in sea-level. Neglecting minor changes, the sea-level appears to have fallen 7 ft. from about 2000 B.C. to the beginning of the Christian era, and to have remained relatively stable since then. The changes correlate favourably with transgressional periods recorded in Europe. The present rise of 8–9 in. a century may well be only a minor fluctuation in what is now an essentially stable ocean-level.

Hatfield Technical College

As from September next Hatfield Technical College will be renamed Hatfield College of Technology. Three departments of engineering have recently been formed, namely: the Department of Mechanical and Aeronautical Engineering (head of department, D. R. Samson); the Department of Industrial Engineering (head of department, J. D. Smith); the Department of Electrical Engineering (head of department, B. F. Gray). It is also announced that Dr. R. F. Robbins has succeeded Dr. Fred. Lester as head of the Department of Science.

The Institute of Metals

The Council of the Institute of Metals has made the following awards of medals for 1961: *Institute of Metals (Platinum) Medal*, to Major C. J. P. Ball, chairman of Magnesium Elektron, Ltd., in recognition of his outstanding services to the non-ferrous metal industries and, in particular, to the magnesium industry; *Rosenhain Medal*, to Dr. P. B. Hirsch, of the Cavendish Laboratory, Cambridge, in recognition of his outstanding contributions in the fields of physical metallurgy and metal physics.

The following have been elected officers and members of the Council of the Institute of Metals: *president*, Prof. H. O'Neill (head of the Department of Metallurgy, University College of Swansea, University of Wales); *past-president*, Sir Ronald Prain (chairman and president, Rhodesian Selection Trust, Ltd.); *vice-presidents*, Mr. W. F. Randall (deputy chairman and managing director, Telcon Metals, Ltd.) and The Right Hon. The Earl of Verulam (chairman, Enfield Rolling Mills, Ltd.); *ordinary members of Council*, Mr. N. I. Bond-Williams (managing director, the Aston Chain and Hook Co., Ltd.); Prof. A. H. Cottrell (Goldsmiths' professor of metallurgy, University of Cambridge); and Mr. G. A. Rider (deputy managing director, Birmid Industries, Ltd.).

University News: Birmingham

The Ford Foundation has given the University a grant of 440,000 dollars to support a programme of research extending over the next fifteen years in the Department of Anatomy into the reproductive physiology of monkeys. The Department has been made the recipient of this benefaction because, under the direction of Sir Solly Zuckerman, work of this nature has been one of its main interests for many years.

London

THE following titles in respect of the posts occupied have been conferred: Sir William Collins professor of anatomy in the University on Mr. G. W. Causey (Institute of Basic Medical Sciences); reader in veterinary physiology on Dr. F. R. Bell (Royal Veterinary College); reader in anatomy on Dr. A. S. Breathnach (St. Mary's Hospital Medical School); reader in geography on Dr. E. H. Brown (University College); reader in clinical rheology on Mr. D. G. Melrose (Postgraduate Medical School of London); reader in chemical engineering on Dr. J. W. Mullin (University College).

Southampton

SIR BASIL SCHONLAND, director of the Atomic Energy Research Group, Harwell, has been elected junior pro-chancellor in succession to the late Sir Henry Tizard.

"Fatigue Straining of Copper Whisker"

DR. EDWARD EISNER has written with reference to the communication in *Nature*, 188, 1183 (1960) directing attention to some misprints. "The units of stress in paragraph 1 should be kgm./mm.^{2}. The second sentence of paragraph 3 should contain the phrase '. . . with the whisker encastered in diphenyl carbazide glass . . . ' instead of the present phrase. This is intended to convey that a part of the whisker at one end was embedded in the glass, so that that portion of the whisker was rigidly held, approximately normally to the surface of the glass. The second part of the last sentence of paragraph 7 should read, ' . . . it afterwards withstood 10^6 cycles at 0–1·1 per cent and 10^6 cycles at 0–1·7 per cent strain, and it kinked after 10^5 cycles at 0–2·3 per cent strain'. "

Announcements

MR. DENZIL FREETH, member of Parliament for Basingstoke, has been appointed Parliamentary Secretary to the Minister for Science, and will answer in the House of Commons for the Lord President of the Council and the Minister for Science.

SIR EDWARD APPLETON has been awarded the Gunning Victoria Jubilee Prize for the period 1956–60 of the Royal Society of Edinburgh for his distinguished contributions to ionospheric and radio physics.

MR. BRIAN G. ROBBINS, secretary of the Institution of Mechanical Engineers, is to retire on June 1 and will be succeeded by Mr. Kenneth H. Platt, at present deputy secretary of the Institution. Mr. Platt, who is a graduate of the University of Glasgow, has been associated with engineering education and training, both in industry and with the Ministry of Education.

THE French Government is offering again this year scholarships for further study or research in France. The grants will be given for periods of 3–10 months during the academic year 1961–62. Candidates should hold a degree or equivalent qualification. Applications from graduates in science or medicine will be particularly welcome. Application forms and further information can be obtained from the Scientific Office, French Embassy, 22 Wilton Crescent, London, S.W.1. The closing date for application is March 16.

THE Trustees of the Ramsay Memorial Fellowships Trust will consider in June applications for a Ramsay Memorial Fellowship for advanced students of chemistry. The value of the fellowship will be £700 per annum, to which may be added a grant for expenses of research not exceeding £100 per annum, and it will normally be tenable for two years. Completed application forms must be received by the Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London, W.C.1, not later than April 17.

THE Institute of Physics and the Physical Society is arranging a conference on "Palæomagnetism", to be held at King's College, University of Durham, Newcastle upon Tyne, during April 20–22. Further information can be obtained from the Administrative Assistant, Physics Department, King's College, Newcastle upon Tyne.

AN international symposium, entitled "Host Tumour Interactions", is to be held at the Henry Ford Hospital in Detroit, Michigan, during May 18–20. Further information can be obtained from Dr. Michael J. Brennan, Oncology Division, Secretary, Symposium on Host Tumour Interactions, Henry Ford Hospital, Detroit 2, Michigan.

THE Unesco South Asia Science Co-operation Office is organizing a regional seminar on "Scientific Documentation", during March 7–16, at the Indian National Scientific Documentation Centre, National Physical Laboratory, Hillside Road, New Delhi. Further information can be obtained from Unesco South Asia Science Co-operation Office, 21 Curzon Road, New Delhi.

THE Council for British Archæology is organizing a conference on "Prospecting in Archæology by Geophysical Methods" in collaboration with Dr. J. C. Belshé, Department of Geodesy and Geophysics, University of Cambridge. The conference will be held in the Institute of Archæology, 31–34 Gordon Square, London, W.C.1, during March 4–5. Further information can be obtained from the Council for British Archæology, 10 Bolton Gardens, London, S.W.5.

A DISCUSSION conference on "Science and Education" is being arranged by the Association of Scientific Workers, Association of Teachers in Technical Institutions, Association of University Teachers, National Union of Teachers and Science Masters' Association, and will be held at the Mahatma Gandhi Hall in London on March 11. Correspondence in connexion with the conference should be addressed to the Conference Secretary, c/o Association of Scientific Workers, 15 Half Moon Street, London, W.1.

A ONE-DAY symposium on "Computers for the Chemical Engineer" is to be held in the University of Birmingham on March 28. It is being organized by the Graduate Section of the Birmingham University Chemical Engineering Society and the Graduates and Students Section of the Institution of Chemical Engineers, Midlands Centre. Further information can be obtained from the Registration Secretary, Department of Chemical Engineering, University, Edgbaston, Birmingham, 15.

WRITING with reference to the communication entitled "Spontaneous Cytomegalic Inclusion Body Disease involving Lacrimal Glands of Caesarian-derived (so-called) Pathogen-free Rats", in *Nature*, 188, 506 (1960), Dr. Hans Meier states the animals used were not ". . . raised in the Roscoe B. Jackson Laboratory", but in the laboratories of the Children's Cancer Research Foundation, Boston, where the work was carried out

THE 1961 EXHIBITION OF THE INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY

THE forty-fifth Annual Exhibition of Scientific Instruments and Apparatus held in the Old and New Halls of the Royal Horticultural Society in London during January 16–21 was the first since the amalgamation of the Institute and the Society. By continuing in its established tradition, it has incidentally managed to conform with ancient precedent, for the sixth King James of Scotland also suffered no change of character on becoming the first of a united body. It was propitious that the activities and publications of the Institute and Society were displayed conspicuously on an attractively arranged stand, and many visitors must have been glad of this opportunity to make a closer acquaintance with their host. From the point of view of learning about new developments, it was a very good exhibition. The footnote 'demonstration' in the catalogue usually meant not just that a device would be working, but that an experiment to explain its principle or its uses had been mounted. Things progress so fast nowadays that even the term 'trend' would be out of place (if it has not already been registered to name a detergent, anyway); and the uninitiated can only hope to sense what is more like a rate of increase of acceleration. This seemed steepest in fast-operating elements for computers, instrumentation for thermonuclear research, and micro-wave amplifiers, all directed towards the future; and in physics applied to archæology, using nuclear and other techniques for raking up the past.

Several stands exhibited devices incorporating the new Esaki or 'tunnel' diode, made of germanium antimonide or indium antimonide; this operates by a quantum-mechanical tunnelling across the energy gap at a p–n junction, and its chief virtue is high speed. Its uses in logic and memory circuits and for amplitude–time discrimination were shown. The Royal Radar Establishment illustrated the behaviour of a tunnel diode in a magnetic field, which alters the energy gap and reduces the current. Microminiaturized solid circuits, and also circuits built up from thin films, were displayed. Among new ceramic materials were those designed for capacitors, high-temperature ceramics of the silicon nitride and silicon carbide type, and ceramic elements for electroluminescent panels.

Thin magnetic films, using material of the permalloy type, are made for information storage and millimicrosecond switching elements. Two firms demonstrated apparatus for determining the hysteresis loops of thin films, and for observing the domain boundaries visually by means of the Kerr magneto-optical effect. The General Electric Company showed how the easy direction of magnetization can be determined, and also a device for testing magnetic-film matrix elements under simulated computer conditions.

How to deal with the 14-MeV. neutrons resulting from the (D,T) reaction is one of the problems of the thermonuclear investigators, and three neutron generators producing 14-MeV. neutrons by bombarding tritium-containing targets with 100-kV. accelerated deuterons were displayed, for either pulsed or steady operation. These will enable studies of this interesting product of the (D,T) reaction to be made at high flux densities without having to operate *Sceptre* and such-like as sources. Associated Electrical Industries, Ltd., of Aldermaston, showed a data-processing system for thermonuclear research, following the fluctuations of an unstabilized discharge in a glass torus, intended to study what goes on in *Sceptre* itself.

Amplification of various kinds was featured in many exhibits. Five-stage optical image intensifiers, using secondary emission from thin-film dynodes and amplifying up to 100,000 times, represent one aspect; and there were many micro-wave low-noise and other amplifiers. Standard Telephones and Cables, Ltd., illustrated the principle of the parametric amplifier by a mechanical analogue; and there was a parametric amplifier for ultra-high frequencies which operated after frequency conversion down to the X-band. I learned from a physicist working in this field that the maser of the future for satellite and space work is the travelling-wave tube—of the immediate future, that is. Production types of travelling-wave tubes were shown, and the Services Electronics Research Laboratories had a high-power version which operated at about 5 kW. This establishment also had a cyclotron wave-amplifier (Adler tube); and Associated Electrical Industries, Ltd., featured what was called a quadrupole electrostatic cyclotron wave-amplifier, which has not so far reached the stage of being a low-noise device.

Fundamental physics was exhibited in the demonstration arranged by the Atomic Energy Research Establishment of the Mössbauer effect, in which the γ-rays emitted by one transition in an excited iron-57 nucleus are absorbed by a different transition in an iron-57 target moving at a suitable relative velocity, the resonance being displayed on a velocity spectrometer. Among the exhibits from the National Physical Laboratory was a new absolute radiometer, determining radiant power in terms of electrical power. What might almost be called 'unfundamental physics' was shown in a piece of intriguing hydrometry, which used a thin-walled transducer vibrating within a liquid to determine the density of the liquid to better than 1 per cent—valuable for the particular kind of instrumentation that it was aimed to do, of course, but a long way round in principle.

Magnetometry was represented by proton-resonance magnetometers; one by Mullard, Ltd., adapted to control the oscillator at what the description consistently and nostalgically called the "Lamour frequency", and another as part of the comprehensive exhibit of the Research Laboratory for Archæology and the History of Art, Oxford, where one would have liked to spend a good deal longer learning about the highly ingenious stratagems used to detect buried buildings and pottery by measuring the small changes they produce in the ambient magnetic field and its vertical gradient; it was amazing to learn that the real source of this change is in many cases the result of a slight alteration in the magnetic properties of clay produced by firing. The Signals Research and Development Establishment demonstrated a rubidium-vapour magnetometer, which is best described as the optical-pumping (or 'photon-spin-pumping') device working in reverse. The effect of a magnetic field, which separates the otherwise degenerate spin-levels by an extent proportional to this field if it is small, is used to measure the strength of the field. A perpendicular radio-frequency magnetic field is adjusted to the energy difference between the pair of levels which have been population-inverted (that is, to the Larmor frequency when the system starts to operate maser-wise, a state that is detected by the strong absorption of the pumping beam of circularly polarized rubidium D_1 light). Thus, instead of tuning to a given radio-frequency by altering the field, the system is tuned to the ambient field by altering the frequency. This is sufficiently sensitive for geomagnetic observations, and may possibly be applied to aerial and space magnetic surveys; indeed, the actual apparatus was too sensitive to work in the disturbing fields of the Hall, and was planted out on the Westminster School playing-field.

These are the impressions of a day's visit; a proper appreciation would have needed the full five days, and some considerable preparation beforehand. There was much that I missed, and one highlight in the form of a miniature lighthouse with a brilliant source that I even failed to identify properly. The exhibits from various research laboratories of the Department of Scientific and Industrial Research were representative of the wide variety of tasks they undertake. The Science Museum had two exhibits, one ancient and one modern, and there were six contributions from university departments; it would add further facets to a brilliant occasion if we could see, among all the new tools for research and development, more of the new apparatus designed at the universities for their practical classes and demonstrations, and at the Science Museum for the instruction of the general public.

Evening discourses were given during the Exhibition by F. S. Burt, on "Hydrodynamics Research"; by Dr. G. E. R. Deacon, on "The Physics of the Ocean"; and by Dr. F. P. Bowden, on "Some Physical Problems in Travelling at Supersonic Speed".

G. R. NOAKES

CALORIE BALANCE IN MAN

THE one hundred and thirty-seventh meeting of the Nutrition Society was held in the Middlesex Hospital Medical School on December 17, and took the form of a symposium on "Calorie Balance in Man". Perhaps it was optimistic to expect a symposium, continuing from 10.15 a.m. to 4.30 p.m. on the Saturday a week before Christmas, to be popular. In fact, it was a well-attended meeting with an audience of more than 100. The chairman was Sir Charles Dodds.

Not so many years ago the subject of "Calorie Balance" would have been an unlikely topic for such a symposium. Interest in calories, at least in relation to human energy, was at a low ebb scientifically and a rather naive idea was prevalent that little new remained to be discovered in this field. Far from this being the case, a most notable impression gained from the speakers at this symposium was the great dearth of reliable relevant information.

The lack of information and the misconception about many aspects of human energy metabolism were mentioned in relation to some of the "Basic Physiological Factors affecting Calorie Balance", the title of the paper by Dr. J. V. G. A. Durnin (University of Glasgow). Part of the complexity of 'calorie balance' arises because of the difficulty of assessing methodological accuracy; there is no sure way of knowing whether the intake of calories or the expenditure of energy represents the 'normal' or usual state for the individual subject. He, or she, may be eating more or less, during the experimental study, than would normally be the case, or the level of energy expenditure may be altered. Since it is extremely difficult under field conditions to carry out such measurements over a period of more than a few days, theoretically one possible way of finding a departure from the normal would be to measure a change of weight in the subject. Part of this paper and a large part of the discussion were devoted to the balance of weight, and Dr. Durnin showed results for young men, weighed under highly standardized conditions, where their gross body-weight changed by up to 3 lb. (1·4 kgm.) from day to day. Such changes, presumably almost entirely due to alterations in the fluid content of the body, make the weighing of subjects quite worthless in regard to the balance of energy.

Other factors dealt with were exercise (so often discussed emotionally rather than rationally), climate, age (growth in the young and inversion of growth in the old) and the efficiency of utilization of food by the body.

That most of us do not normally attain balance of energy from day to day was shown clearly by an analysis on the results for 69 individuals, where only 4 subjects had a significant correlation between their intake and output of energy; our 'appetite' is a fickle mechanism.

Calorie balance is probably a long-term control, which may be effected by centres in the hypothalamus responding to changes in the fat depots of the body and then influencing the general level of food intake and perhaps even of bodily activity. In this control, the reticular activating system of the brain stem, the limbic system and the hypothalamus may act together. This was suggested by Dr. G. C. Kennedy (University of Cambridge) in his paper "Central Nervous Regulation of Calorie Balance". The importance of exercise was stressed, both in relation to man and to studies on animals; much work on rats or mice is not comparable because of varying levels of physical activity resulting from restrictions imposed by some types of animal cages. Similarities between a 42 stone (267 kgm.) obese patient and experimentally obese rats were shown by both the astonishing hyperphagia and also lack of resentment or discomfort when food was restricted. They were also alike in their inactivity.

It has been the custom, at certain levels of medical therapeutics, to treat obesity as if it were primarily an endocrine disorder and give the patient mixtures of tissue extracts from various endocrine glands.

The traditional blame attached, by many clinicians, to the endocrine system in the causation of obesity was repudiated by Dr. Raymond Greene (London) in "The Effects of the Endocrine System on Calorie Balance in Man". The several endocrine glands were considered in brief, abnormalities of some of them being associated with modifications in the water content of the body; occasionally obesity occurs concomitantly with endocrine pathology but rarely, he thought, is hormonal disorder of any great importance. Dr. Greene suggested that a more interesting discourse, rather than the prescribed one, might have been on "the influence of nutrition on the endocrine system".

Within recent years some changes in outlook on the nature of fat metabolism have been apparent. It has been suggested, although not always with convincing experimental evidence, that the fat depots of the body are far from inactive tissues and indeed are continuously being mobilized. Their nerve supply is stated to be extensive, although most histologists appear to have some difficulty in finding many nerve endings, as opposed to nerve fibres, in adipose tissue. Prof. A. Kekwick and his colleagues have been engaged in studying the "Metabolism of Adipose Tissue", the subject of a paper by Dr. T. M. Chalmers (Middlesex Hospital, London). He suggested that free fatty acid and not glucose might be the principal energy substrate, because of its rapid turnover in the plasma. The fat-mobilizing substance, found in the urine of fasting men, might have some physiological role; its release in the urine apparently depends on carbohydrate metabolism, this being demonstrated in one obese patient treated with equicalorific diets containing varying amounts of carbohydrate.

The second half of the meeting began with a paper on "Energy Expenditure and Calorie Intake in Young Men" by Dr. O. G. Edholm (Medical Research Council, London). In a series of 6 surveys, on 54 Army recruits, he had found variations in energy expenditure of from 2,200 to 6,100 kcalories/day and of intake from 671 to 8,750 kcalories/day, with means of about 3,800. The lack of agreement between calorie balance and the weight balance of the body was again stressed, when these were measured over periods of a few days. There is not sufficient information at present to determine the effect, if any, of severe cold on calorie balance. Work carried out in the Antarctic has shown that men put on weight in the winter and lose weight in the summer; this might be due to climatological factors, but equally well might reflect different social circumstances.

The amount and the rationale of the extra "Calorie Requirements in Pregnancy" were discussed by Dr. A. M. Thomson and Dr. F. Hytten (University of Aberdeen). From a series of several hundred healthy primiparæ studied in Aberdeen, it seemed that an average of about 4 kgm. of fat was added to the mother's tissues by the time pregnancy ended. This is almost certainly physiological, and is required to help the mother in the needs of lactation; however, if breast-feeding is becoming much less common, as appears to be true in Western society, this extra gain in weight may predispose to future obesity. The authors emphasized the small amount of experimental information on the exact increase in food taken during pregnancy and the degree of the changes, if any at all, in the amount of physical activity of the woman; this has a considerable bearing on any extra allowance recommended.

From the interesting paper by Dr. E. M. Widdowson (University of Cambridge) on "Energy Balance in Early Life", new-born babies are frequently subjected to considerable stress by deprivation of food. When this happens, the fat stores of the body are quickly used up, particularly if the baby is required to maintain a high metabolic rate. This will be necessary if the environmental temperature does not fall within the critical range 32-35° C. Babies appear to be as variable in their energy expenditure as adults, and it seems unphysiological to expect all babies to require similar quantities of food.

The discussion of the papers was relevant, interesting and prolonged; the meeting was a most successful one.

J. V. G. A. Durnin

WATER IN AUSTRALIA

MAN'S existence depends on an adequate supply of water, and in Australia, as in few other countries, future development will depend on using water resources to the full. An important step in the utilization of water is the understanding of its properties, and the Victorian Branch of the Royal Australian Chemical Institute found no difficulty in attracting some fifty scientists from four Australian States, whose interests ranged from engineering to biology, to a three-day discussion on this broad topic. The meeting was held at Warburton, Victoria, in late November.

In the opening talk Dr. J. A. Barker reviewed the structure of hydrates and of ice as an introduction to the loosely packed structure of liquid water in which the molecules are prevented from coming into closest proximity by tetrahedrally arranged hydrogen bonds. Each pattern of hydrogen bonds persists for periods of the order of 10^{-12} sec. The theory of Pople, based on this picture, would require each water molecule to have 4 nearest neighbours, 12 next nearest and 36 third nearest, whereas the corresponding figures obtained from analysis of X-ray diffraction data are 4, 11 and 22. Dr. Barker contrasted the anomalous properties of water that result from this structure—the decrease in solubility of gases with increasing temperature, the high negative temperature coefficient of viscosity—with the properties of more normal liquids in which the molecules are closely packed.

Evidence consistent with this structure was presented by Dr. S. D. Hamann, who described some of the effects of pressure on the properties of water and showed how many of the anomalies disappear as the molecules become close-packed at pressures greater than 2,000 atm. Thus with increasing pressure the thermal expansion of water became 'normal', its sound absorption decreased as pressure broke down its structure, and its dielectric constant decreased. Measurements of the conductivity of water under pressures up to 130,000 atm. had produced increases in ionization so that its conductivity equals that of a 5-*N* solution of hydrochloric acid. Pressures of this order are probably reached at depths of 400 km. in the Earth and the enhanced ionization therefore has interesting geophysical implications.

Dr. P. W. Posener showed how microwave spectroscopy could be used to fill in details in the above broad picture.

Considerations of the electronegativity of the oxygen atom in water and hydrates, in both the normal and excited states, led Dr. J. F. Duncan to certain novel and controversial generalizations on the oxidation properties of water. Subjects as diverse as the thermodynamic properties of aqueous ions, the radiolysis of water and the kinetics of aquation reactions could be described semi-quantitatively.

Prof. R. C. L. Bosworth reminded the meeting of the experiments of Nukiyama on the boiling of water, first described in 1934 but disregarded for some fifteen years. A study of boiling produced by a heated platinum wire immersed in water, relating the heat input to the temperature of the wire, revealed three distinct zones of boiling, sometimes with discontinuities between them. The phenomena described by Prof. Bosworth clearly have important practical implications for the design of boilers and heat exchangers. Dr. K. L. Sutherland suggested in discussion that the variation of contact angle with applied potential that had been observed by Frumkin could perhaps be used in controlling boiling. By causing an applied potential to fluctuate at a predetermined rate it might be possible to alternate the periods of bubble formation (large contact angle) with those of bubble release (zero contact angle).

Dr. G. F. Walker described some vermiculite–butyl ammonium ion complexes, their swelling in water, and the intriguing behaviour of their swollen forms. The 10-Å. thick silicate layers, 4 mm.^{2} in area, remained parallel to each other even at interlayer spacings up to 600 Å. The interlayer spacing depended on the concentration and type of cations present and could be varied reversibly under the influence of an electric potential. The phenomenon lacks, as yet, a full theoretical explanation and may have novel applications as a variable molecular sieve.

"Water in Australia" was the topic chosen by Mr. L. R. East in opening the part of the meeting that dealt with conservation. Conservation in Australia poses special problems ; an average rainfall of 16·5 in. compared with the average of 26 in. for all land masses, the large area and small population, and the combined influence of the great variations of rainfall in good and bad years—and of the disastrous reduction of the proportion of rain-water finding its way into the rivers in years of drought. He contrasted the lack of a national water policy in Australia, in spite of significant nineteenth-century contributions, with that initiated in the United States by Theodore Roosevelt in 1901, when he stated that "The reclamation and settlement of arid lands will enrich every portion of our country. . .".

Mr. East discounted the possibility of affecting the climate of neighbouring areas by constructing large inland dams. In this he was supported by Dr. C. H. B. Priestley, who said that, although man had undoubtedly affected weather by his clearing and cultivation of millions of square miles, localized activities were unlikely to have an appreciable effect. He used measurements made over the Mississippi basin to illustrate the balance of water in the atmosphere and showed that up to 90 per cent of the rainfall came from maritime air and only about 10 per cent from continental air.

Mr. E. J. Smith brought the meeting up to date on the progress of rain-making research in Sydney, and pointed out the gaps which exist in the understanding of the nucleation process. In particular, he stressed the possible importance of the particle size of the nuclei as it affected their ability to promote ice-formation and their decay by photolysis and other mechanisms.

Advances in evaporation control with cetyl alcohol were described by Mr. W. W. Mansfield. For large areas of water the alcohol is dusted on to the surface by a grinder-duster, and from the particles a film spreads over the surface. Evaporation, under ideal circumstances, can be reduced by as much as 40 per cent, but the method becomes much less effective as the wind velocity increases. The cost, based on a test at Broken Hill, was one penny per thousand gallons saved.

Two methods of reducing the salt content of water were described by Messrs. R. B. Cooper and L. S. Herbert. Electrodialysis, described by Mr. Cooper, can be used economically to reduce the salt content of water from about 4–5,000 p.p.m. to 3–500 p.p.m. The power needed may be between 9 and 40 kW.hr./1,000 gallons, depending on the salt content and on the flow-rate required. Mr. Herbert discussed some aspects of the distillation of sea-water and suggested possible techniques to improve the efficiency of the process, particularly in increasing heat transfer coefficients. The theoretical minimum energy for any such process is about 3 kW.hr./1,000 gallons, but the practical minimum is about 12 kW.hr./1,000 gallons, a figure which is still far from realization. With known technology it should be possible, on a sufficiently large scale, to produce fresh water for 8*s.* per 1,000 gallons and eventually, with improvements in technique, to reduce this to about 4*s.* per 1,000 gallons.

Mr. J. W. Legge considered the physical-chemical properties of water in relation to the origin and evolution of life, and to L. J. Henderson's views on the fitness of the environment. Knowledge of film stability on natural water surfaces could throw light on current controversy about the kinetics of the synthesis of primitive proteins. He outlined current biochemical views of the splitting of water in photosynthesis and the recombination of hydrogen and oxygen in the aerobic cells.

The movement of water-solute through cell walls was related by Dr. L. R. Finch to the current picture of the membrane. The vesiculation observed in electron micrographs of thin sections, as well as the phenomenon of pinocytosis, involve, of necessity, changes in concentration at the interface. Accelerated metabolism of phospholipids could be shown to take place during such transport processes.

A good deal of discussion followed the contributions of Dr. J. R. Philip and Prof. J. H. Green, which were related to the theory and measurement of ground-water flow. Models for the process are complicated due to changes in the nature of the medium with water content, but Dr. Philip showed how they had been reduced to a tractable form by making use of the basic Navier–Stokes equations. Many were surprised by the high rates of ground-water movement which have been measured by observation of consciously and adventitiously added radioisotopes.

It is pleasing to report on a conference which aroused so much discussion, both formal and less so, and from which so many new patterns of collaboration promise to emerge.

POLITICAL AND ECONOMIC PLANNING IN PAKISTAN

A BROADSHEET recently issued by Political and Economic Planning deals with Pakistan's second Five-year Plan*. After reviewing the first Five-year Plan, it describes briefly the size and objectives of the second Plan and the aims of the various sector programmes, concluding with a brief critique. The fundamental problem for the second Plan is, under severely limiting conditions, to liberate the people from the crushing burden of poverty by ensuring that the rate of growth of the economy exceeds the present 2 per cent annual increase in population. The second Plan, amounting to 1,900 crore rupees, is about 75 per cent larger than the first, a real increase of about 50 per cent, and the methods used are a development of those employed in making the first Plan. Within the various sector programmes, priority is given to schemes already in an advanced state of preparation, or which have already been the subject of foreign government aid; the emphasis is on schemes designed to give quick returns through the better use of existing resources.

Essentially the Plan seeks to make maximum use of the market mechanism, and to employ fiscal and monetary measures rather than direct controls. It adopts a pragmatic approach as between public and private development, with public investment supplementing, rather than displacing, private enterprise, and seeks to develop labour-intensive rather than capital-intensive methods, where a choice is feasible, according to the relative real scarcities of the factors of production. Only a limited call is made on the extra wealth of the community, as the economy progresses, for still further investment, and within the broad priorities of the programmes proposed a substantial element of flexibility has been built into the Plan to meet unforeseeable changes. Of the numerous programmes, those aimed at developing education, health and social welfare services (estimated to cost some 130 crore rupees), like the housing programme, would seem to be out of scale with the needs when judged by contemporary standards and are limited by the resources available. It may be observed that during 1960–65 it is proposed to improve existing social services and to inaugurate schemes for research and training social workers, as well as to attack vigorously such main diseases as malaria, tuberculosis, smallpox and leprosy, to improve greatly the existing schools in East Pakistan, provide more in West Pakistan and extend and improve the universities, as well as extend technical training.

Although the second Plan is regarded as ambitious in the Broadsheet, and requiring devotion, intelligence and hard work if it is to be applied effectively, it is not considered wildly out of scale with the resources, foreign and indigenous, likely to be available. A great effort has been made to improve both the personnel and the systems of administration; a new generation of administrators has been trained and is gradually displacing weak elements from senior positions, while there is some sign that foreign managerial expertize is more warmly welcomed. There is a critical shortage in skilled technicians of all kinds, and a good deal of dilution and improvisation will be needed as well as the full and most effective use of whatever foreign technical services may be available. By and large, it is difficult to find the Plan unsuitable, in any major respect, to Pakistan's needs to-day. There is justification for taking seriously the Government's avowed intention to create a system of democratic government well suited to the conditions of Pakistan. The fact that the second Plan is prepared to tackle the thorny problem of family planning by including a family planning programme of national propaganda, with clinics at all hospitals, dispensaries and maternity centres estimated to cost 3 crore rupees, is regarded as the most hopeful single pointer to the prospect of successful planning in Pakistan.

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* *Planning*, Vol. 26, No. 446 (7th November, 1960): More Planning in Pakistan. Pp. 309–336. (London: Political and Economic Planning, 1960.) 3*s*. 6*d*.

RADIO RESEARCH DURING 1959

THE report of the Radio Research Board and of the Director of Radio Research for 1959 summarizes the wide range of work carried out at the Radio Research Station, Slough, and at the substation in Singapore*.

The report of the Board refers to the recent revision of the structure and terms of reference of its committees in order to ensure that they adequately cover present and future research activities in the radio-field. Considerable emphasis is laid on the importance of radio in space research, and it is planned that in the immediate future about 50 per cent of the effort at Slough shall be devoted to such research. Details of the research work at the Station during 1959 are given in the report of the Director.

Some preliminary work on data obtained during the International Geophysical Year has been done, and this includes an analysis of data on the incidence, at high-latitude stations, of polar radio 'blackouts' and of polar sporadic-*E* ionization. The results show that these phenomena are produced by solar-particle streams incident on the upper atmosphere along arcs which are similar to the precipitation spirals predicted by Stormer fifty years ago.

A study has also been made of the International Geophysical Year data now available for Slough, Singapore and Ibadan on atmospheric radio-noise. The Slough observations provide detailed information on noise characteristics at four frequencies in the range 20 kc./s.–20 Mc./s. The differences between noise-levels at Slough and Singapore are such as would be expected from known differences in meteorological conditions ; but the noise-level at Singapore is not so intense as might be expected from its close proximity to one of the main thunderstorm areas of the world. Work on atmospherics from local storms is providing information on the power distribution with frequency in the lightning flash, and on the extent to which the wave-forms of the radiated disturbances can be explained in terms of the lightning mechanism.

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* Department of Scientific and Industrial Research, 1959: The Report of the Radio Research Board and the Report of the Director of Radio Research. Pp. iv+34+4 plates. (London: H.M. Stationery Office, 1960.) 3*s*. net.

The day-to-day variability in the electron densities of the various ionospheric layers is largely due to the corresponding variability in the wave and particle radiation from the Sun. The derivation of ionospheric indices of solar activity is of special importance for prediction purposes, and the report describes work which has been carried out on such indices for the *E* and *F*2 layers. As part of the International Geophysical Year Calendar Record, *E*-layer solar ionizing radiation indices, *F*2-disturbance indices and polar blackout indices have been calculated for each day of the International Geophysical Year. These daily indices should be invaluable for studies in other International Geophysical Year disciplines.

A rapidly increasing proportion of the activities of the Station at Slough is being devoted to various aspects of space research, and considerable observations have already been made on the direction of arrival of signals from satellites and on the received signal characteristics (Doppler shift, Faraday rotation, telemetry). The station also provides some 100 establishments and observers with predictions, at weekly intervals, of the times and directions of transit of satellites passing within radio-range of observers in the British Isles. Similar work is also being carried out at the Singapore sub-station.

In the radio-measurements and standards field the research and development have been concentrated mainly at frequencies greater than a few hundred megacycles per second. The Station has acted as the co-ordinating centre for the international comparison of milliwatt power-measuring techniques at centimetre wave-lengths, and satisfactory agreement has been found between measurements made with instruments of different types used in the standard laboratories of the United States, Japan and the United Kingdom. Recognition of the possible harmful biological effects of high-intensity electromagnetic radiation at frequencies greater than about 50 Mc./s. has called for the development of reliably calibrated instruments to measure the flux density near high-power transmitters, and the report refers to work being carried out to meet this need.

The study of long-distance ionospheric transmission characteristics, using a sweep-frequency pulse technique, has continued and been extended to a 6,700-km. path between Ascension Island and Slough. It is found that during the daytime the modes of transmission are always very complex because of reflexions from tropical sporadic-*E* ionization, and the sunset deterioration and fading of signals from stations in equatorial latitudes is associated with the break-up of this sporadic ionization at such times. Work on the lateral deviation of signals from Japanese short-wave stations shows that there are often periods when the signals depart significantly from the great-circle path and that propagation by way of auroral reflexion is probably a common feature of night-time propagation over these paths.

Tropospheric radio-wave work includes studies of scatter propagation, delay times on microwave links and the refractive properties of the lower atmosphere. Extensive signal-level, gain in height, fading and diversity measurements have been made over distances from 130 to 400 km. ; but it would appear that no completely satisfactory explanation of troposphere scatter propagation is yet available. The band-widths which can be used in short point-to-point microwave communication links are limited by multi-path propagation, and equipment is being developed at Slough to enable accurate detailed work on the signal-delay times to be measured. The refractive properties of the lower atmosphere are being investigated by means of airborne refractometer measurements, by radar sounding and also by using pressure, temperature and humidity data obtained from a balloon-borne wired-sonde.

Research at the Station on semiconductors has been mainly on transistors and their application. Microwave measurements on certain ferrite–aluminates in a resonant cavity and on transmission phenomena in wave-guides containing these materials has continued.

This 1959 report is of particular interest in that it is the last annual report of Dr. R. L. Smith-Rose, consequent on his retirement from the post in September 1960.

W. J. G. BEYNON

SAFEGUARDING HEALTH IN LONDON

THE annual report on the work of the Scientific Branch, Public Health Department, London County Council, has been prepared by Dr. S. G. Burgers, scientific adviser to the Council*. The Department has 64 members of whom 34 are graduates, and the work covered by them during 1959 varied from routine analysis of foodstuffs—still very necessary—to investigating the properties of cleansing materials used in kitchens and laundries. Much research of a fundamental nature is carried out and opens up many possibilities for economic exploitation. The Council is to be commended for the high quality of the investigations carried out by this section of its staff.

Particularly valuable research was connected with the treatment of sewage. How to produce sulphur from sulphate-enriched sludges was investigated by one team, and valuable information was obtained about the cost and practicability of the process, if conditions of national emergency should render its use necessary. Because of the long duration of these experiments, useful information was also accumulated about the effect of sulphide sludges on metal and other components of the experimental plant. Metals were found to be singularly free from corrosion—stainless steel was as bright after three years exposure as it was initially, mild steel and cast iron (partly protected by calcium carbonate scale) were scarcely affected and steel bolts immersed for eighteen months could be unscrewed with ease. Brass and lead, except for a slight discoloration due to a sulphide film, were almost unaffected, but the zinc coating on the galvanized iron heating coils had completely disappeared, although this may have been due to electrolytes other than sulphides. Rubber was apparently unaffected.

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* London County Council. Public Health Department—Scientific Branch. Annual Report of the Scientific Adviser for the year 1959. Pp. 46. (London : London County Council, 1960.) 1*s*. 3*d*.

Experiments were also continued on the final disposal of concentrated foam liquids, with particular reference to stripping by means of activated carbon. Aqueous solutions of 1,000 p.p.m. of sodium tetrapropylene benzene sulphonate were passed through a column of 1 in. diam. and 8 in. long, containing 100 gm. of activated carbon. The carbon was soaked in water before use, and care was taken to maintain the liquid level half an inch above the carbon column. During continuous flow the activated charcoal showed almost complete adsorption of the detergent present in the solution in the early part of the test; it was capable of extracting about 90 per cent of the detergent after it had adsorbed 27 per cent of its own weight of surface-active material and could still extract 50 per cent after adsorbing 38 per cent of its own weight. In a later experiment, a solution of 1,000 p.p.m. of sodium tetrapropylene benzene sulphonate in the final effluent from an activated sludge plant was passed through a column containing 100 gm. of activated carbon. A gauze pad was used to intercept suspended solids before the liquid reached the activated carbon in order to avoid choking the interstices in the column. Although adsorption was good, there was evidence that the carbon failed more quickly than when aqueous solutions were used, probably because of adsorption of other constituents of the effluent.

As activated carbon is expensive, attention was given to the possibility of regenerating the material. Attempts to displace the surface-active materials with solvents had only a limited success, and acidification of the dried exhausted carbon, followed by extraction with trichlorethylene, resulted in the removal of only a small proportion of the synthetic detergent in the form of its sulphonic acid.

The most promising method of disposal of condensed foam liquids appeared to be by spraying the foam with surface-activated sludge and allowing the solid content of the material to settle. In the process, the detergent concentration of the supernatant liquor might be expected to be reduced from about 500–600 to 30–40 parts per million, which could easily be treated by admission to primary sedimentation tanks.

Continuing a long-term investigation into the incidence of air pollution, volumetric instruments again indicated a reduction of 7 per cent in the average concentration of smoke in the air of London during the year, and this progressive tendency for improvement has continued since the inception of the Clean Air Act at the beginning of 1957. The sulphur dioxide concentration, for the first time, showed a similar reduction. The improvement was mainly attributable to exceptionally good weather in December, in which the average smoke concentration was only half the usual figure. It is probable that mild weather at the end of the year resulted in reduced domestic coal consumption, and, if this is true, the improvement may not be permanent. It is of interest that during the unusually fine summer the degree of pollution, though naturally low, was very similar to that of the previous cold, wet summer.

A five-month investigation into conditions at Camden Depot was completed, showing that a house adjacent to the railway was subjected to concentrations of black smoke and sulphur dioxide which were on the average 102 and 43 per cent respectively higher than the general London-levels. With the wind in certain directions, the average concentrations rose to at least 200 and 100 per cent respectively above the same London-levels. The situation is expected to improve with the gradual replacement of steam by Diesel locomotives.

RESEARCH IN THE SOCIAL SCIENCES

PRIORITIES for research in the social sciences in industry have been indicated by A. B. Cherns, head of the Human Sciences Section, Department of Scientific and Industrial Research (*Personnel Management*, 42, No. 353; December 1960). In the field of ergonomics, for example, Cherns suggests that the man–machine relationship is a vital aspect of the study of man in industry. The social implications of the man–machine system are such as to justify more attention from social scientists perhaps as members of mixed ergonomic and sociological teams. Ergonomics is a field in which practical applications are easy to appreciate.

The development of adequate criteria of industrial relations should receive a high priority. Have the criteria also to develop indicators? Could the industrialist be advised what chart he should put on his wall to give him a guide to the industrial relations formulated in his plant? Until this be done, the claim to have extracted the full practical value from research cannot be substantiated. A wide comparative study would seem to be required.

The approach of Flanders and others, which aims at developing a study of industrial relations in all its aspects—economic, legal, psychological, sociological and historical—may well provide the stimulation that is needed both to give point to and to encourage wide comparative studies.

There has been an uneasy tug-of-war between interpretations of workshop behaviour based on views of the man in the workshop as an economical and rational animal and those based on views of him as a nexus of social roles—the economic and the sociological points of view. More accurate and systematic observation is a high priority—but not of the extended, reporting type with platitudinous conclusions.

More analysis of the pressures on the individual in industry is required; in particular, incentive payment systems, methods and standards of inspection, the structure and organization of management, and delegation of responsibility.

The study of individual responses to pressures is as important as the study of the pressures themselves.

The new enthusiasm for industrial sociology has tended to obscure the older studies of individual differences. It may now be time to look at these again in the light of better understanding of the social pressures involved.

Examination of status systems in industry is not new. Nevertheless, there is need to know more about the extent to which the use of the special knowledge and ability of the technician or technologist is hindered by the status systems in industry, particularly the caste system dividing management from technical grades. This is in need of overhaul based on sociological understanding.

The work of Simon and his associates in the United States has shown what can come from a rigorous analysis of some aspects of decision taking. There is a point in each organization at which there is someone who has the necessary authority to take a particular decision. Also located in this organization is someone who has the knowledge for the best decision to be taken. The problem is how to get these two points to coincide: How can effective authority coincide with effective knowledge?

Industrial relations generally means relationship of management to labour. Equally important for the health of any concern is its management/management relations. These may not burst into active conflict, but latent conflict is continuous and may be dangerous. This conflict is neither altogether avoidable nor altogether bad; every time a functional organization is set up a conflict of interest is created. Each functional department tends to seek to maximize what, from the point of view of the organization as a whole, needs to be optimized. For example, a production department will naturally have maximum production as its goal; an inspection department will naturally tend to seek maximum quality or minimum errors. From the point of view of the concern as a whole, a compromise between these conflicting aims has to be achieved. Less obvious but equally profound conflicts of aim will occur between the personnel department and the department of the accountant or financial controller. The problem is not how to resolve this conflict but how best to use it and keep it within bounds.

T. H. HAWKINS

TRAINING ENGINEERING GRADUATES

THE graduate aiming at membership of one of the professional engineering institutions normally undergoes a period of practical training or apprenticeship following the completion of his undergraduate course.

Some three years ago the Institution of Electrical Engineers issued a report entitled "The Training of Graduates" in which the purposes of practical training for graduates were outlined and recommendations for the structure of training courses were made. This report, revised and modified, has now been re-issued*.

The general pattern of training recommended does not differ in its essential features from that described in the earlier report. Training should occupy two years and should comprise basic workshop training, general mechanical and electrical training and what is termed directed objective training. It is emphasized that each phase of the training course should be carefully planned and supervised and that the graduate in his practical training should continue to experience something of the intellectual stimulus and of the urge to intensive individual effort which are inherent in academic studies.

Separate sections of the report deal with schemes of training conducted in a works making heavy electrical plant, a factory for light-current equipment and in an operating organization, such as an electricity supply undertaking or a telecommunication service.

While the majority of electrical engineers study, as undergraduates, electrical engineering, a substantial proportion read physics and a few mathematics. The special needs and interests of such graduates in physics and mathematics are met by a scheme of training based on the laboratory but providing for some direct contact with manufacture and development.

There is one important new feature in the recommendations. This is the recognition of training through experience in a staff appointment as an alternative to the traditional form of graduate apprenticeship. This envisages the graduate being engaged as a member of the staff of a professional engineer who will directly supervise his training. The training course will not then conform to any standard pattern, but will comprise the acquiring of experience on a series of projects. These will be so chosen as to provide in the aggregate a range of experience equivalent to that provided by the normal graduate apprenticeship. Great stress is laid on the personal responsibility accepted by the engineer who undertakes to provide this type of training.

* Institution of Electrical Engineers. The Training of Graduates: a Report of the Joint Committee on Practical Training in the Electrical Engineering Industry. Pp. 34. (London: Institution of Electrical Engineers, 1960.) 2*s.*

POLIOMYELITIS

SINCE the last World Health Organization specialist Committee on Poliomyelitis met in 1957 not only have inactivated poliovirus vaccines come into widespread use but also live attenuated vaccines have been submitted to intensive study and, in some countries, have already been employed on a scale that could scarcely have been envisaged three years ago. These developments and the problems they raise are fully reviewed in the third report of the Committee (Expert Committee on Poliomyelitis, Third Report. World Health Organization: Technical Report Series, 1960, No. 203; 53 pages. Price: 3*s.* 6*d.*, 0.60 dollars, Sw. Fr. 2. Also available in Spanish and French).

In most countries where inactivated vaccines have been widely used the protection obtained has been of the order expected on the basis of controlled field trials. Manufacturing and testing problems appear to have diminished with increasing experience, but recent attempts to manufacture polyvalent vaccines containing poliomyelitis antigen have raised some special problems. Experience with such multiple antigens is still too limited for their value to be assessed.

The safety of live poliomyelitis vaccines appears to have been satisfactorily demonstrated in a number of areas where they have been used on a large scale, and, in all except two of these areas, the incidence of poliomyelitis has fallen. It is still too soon, however, to evaluate the long-term effectiveness of live vaccines. Criteria for evaluating the safety and effectiveness of such vaccines are formulated in the report. In this connexion, further research on virus markers and on the degree of viræmia produced by different strains is urgently needed. The capacity of the various strains to spread contacts in the community is also a matter needing investigation. The Committee proposed criteria for the selection of suitable strains and for grading various strains of the three sets of vaccines in use. It also gave attention to the possible contamination of live poliovirus vaccines by simian viruses, and recommended the study of their pathogenicity and methods of differential inactivation.

The choice of inactivated or live virus vaccine for large-scale immunization programmes will depend on the epidemiological, social and economic circumstances of the country concerned. The Committee examined the advantages and disadvantages of the two types of vaccine from these aspects and suggested a number of considerations to be kept in mind by public health authorities when deciding which policy to adopt. Emphasis is laid on the importance of serological surveys and surveillance for the efficient conduct of immunization programmes. Finally, attention is directed to the increasing prèvalence of other enterovirus infections (Coxsackie and ECHO viruses) which cause a wide range of clinical manifestations sometimes resembling poliomyelitis. Continued research is needed in this field, especially to detect any increase in the severity of the clinical manifestations in special groups, for example, new-born infants.

UPPER WINDS OVER THE WORLD

THE appearance of *Geophysical Memoir* No. 103 from the Meteorological Office will be welcomed by all who use upper-wind data, whether for geophysical or aviation purposes*. This supersedes *Geophysical Memoir* No. 85, which had the same title, and was published ten years ago, but has long been out of print. The present publication is based, almost entirely, on data for the five-year period 1949–53. Though the International Geophysical Year will have provided a wider coverage to greater heights, it will always be useful to have a detailed analysis of the epoch 1949–53 for reference and comparison, particularly as the information is given for the months of January, April, July and October, rather than as averages for the three-monthly periods of four seasons. This latter procedure was adopted in *Geophysical Memoir* No. 85 on account of paucity of data, but results in excessive smoothing. Average contours are given at the pressure-levels 700, 500, 300, 200, 150 and 100 mb. (approximately 3,000–16,000 m.) and also the average winds are given by diagrams of streamlines and isotachs. The North Polar regions have circumpolar charts for the regions north of 65° N., but no data for the southern hemisphere are given south of 65° S. This is because of the absence of data, for there are large areas of the southern oceans without observations and there must have been a great deal of guess-work there.

Meteorologists will be especially interested in the vertical cross-sections of average zonal wind and potential temperature given for the longitudes 140° E., 75° E., 10° W., 75° W., 130° W. and 180° W. for the same months. These illustrate very dramatically the complexities of atmospheric motions.

In order to estimate the probable wind at any point, it is also necessary to know the standard vector deviation, as well as the average wind. Charts of standard deviation are not provided, but it is promised that they will be given in a later publication.

A. W. BREWER

* Air Ministry: Meteorological Office. *Geophysical Memoirs*, No. 103: Upper Winds over the World, Parts 1 and 2. By H. Heastie and P. M. Stephenson. Pp. 1+217. (M.O. 631c.) (London: H.M. Stationery Office, 1960.) 30*s*. net.

DIRECTION OF THE GEOMAGNETIC FIELD DURING THE TRIASSIC PERIOD IN SIBERIA

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AN earlier detailed study of the Deccan Trap formation[1,2] revealed, wherever samples were taken, the existence of a stable remanent magnetization making large angles with the ambient field. One explanation has been that India was in middle latitudes of the southern hemisphere during the late Cretaceous or early Eocene. This would also conform broadly to palæogeographical reconstructions offered in the literature, according to which India once formed part of a southern super continent the constituents of which later dispersed.

Such an interpretation prompts the question to what extent the rest of what is now Asia participated in the hypothetical northward drift of India. It

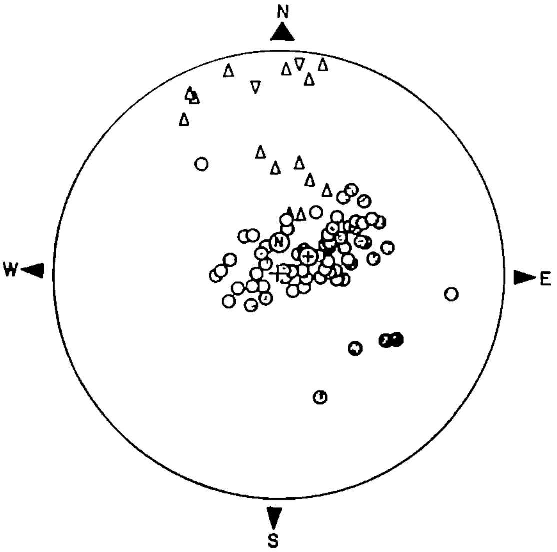

Fig. 1. Polar equal-area plot of the remanent magnetization. Δ, data for specimens from site *C*, N. pole pointing down; ∇, site *C*, N. pole up; ○ all other sites, N. pole down; ⊕, mean direction for all samples, omitting site *C*; *N* in circle, theoretical dipole field at the sampling locality

Table 1. MEAN REMANENT MAGNETIZATION OF SAMPLES FROM SITES *A*, *B*, *D* AND *E*

S	*N*	Declination	Dip	α_{50}	α_{95}	Intensity (c.g.s.)
67	26	N. 62° E.	+76° down	4·0°	8·5°	$(1{\cdot}87 \pm 1{\cdot}14) \times 10^{-3}$

S, number of specimens measured; *N*, number of samples; α_{50}, α_{95}, radii of 50 and 95 per cent circles of confidence, using Fisher's method. All averages are based on samples (*N*), not specimens.

seemed that a promising source of evidence might reside in the palæomagnetism of rock formations north of the Himalayas; in particular, the vast Siberian land mass offers manifold opportunities.

Consequently, in 1957, a request for some oriented samples from Siberia was made to Prof. V. V. Fedinsky of Moscow, then attending the eleventh Assembly of the International Union of Geodesy and Geophysics in Toronto. This was arranged without delay, and in 1958 the University of Alberta in Edmonton received a collection of trap rock from the northern part of the Enisei River system.

The samples are from five exposures in the valleys or basins of the Lower Tunguska, Severnaya and Dudinka Rivers, located as follows (Fig. 2): Site *A*, 66·0° N., 89·0° E.; Site *B*, 69·3° N., 88·0° E.; Site *C*, 66·5° N., 90·1° E.; Site *D*, 66·1° N., 89·0° E.; Site *E*, 66·5° N., 89·1° E. The formations are of Lower Triassic age, range in type from gabbro-diabase to diabase, and at all sites are steeply inclined to the horizontal. Gabbro and gabbro-diabase are elsewhere[3] described as typical of the thick intrusive bodies which characterize the Siberian Traps in this region.

Samples were cut into 1–3 cylindrical disks, which will be referred to as 'specimens'. Direction and intensity of their magnetization were determined with a simple astatic magnetometer, mounted inside a hut belonging to the University of Alberta in Calgary, where we carried out the measurements during summer, 1960.

The stereogram (Fig. 1) shows results for all 83 specimens cut from 32 samples. With few exceptions, the vector extremities of the disks from sites *A*, *B*, *D* and *E* form part of a fairly dense central cluster. In contrast, the plot of points from site *C* is anomalous, and as their scatter exceeds that for any other site, the results were considered unreliable, and hence rejected. The resultant of 26 normalized vectors, all being specimen averages, then corresponds to a west-south-west—east-north-east axis dipping downward steeply towards the east (Fig. 1, Table 1).

In assessing whether this is a true record of the local field direction during Triassic times we make the usual assumption, which seems well supported by theory and some observation, that over periods of 500-1000 years the geomagnetic axis is that of a dipole coaxial with the spin axis. The magnetization of some specimens from each site had been provisionally determined in 1958, but was essentially the same after two years random storage. However, long-term stability may be inferred more compellingly from the data themselves, whenever the vectors form a compact group the mean direction of which is in misalignment with the present local, or dipole, field. The observed angle of deviation, though not large, was taken as significant, for it exceeds the semi-angle of the 95 per cent cone of confidence surrounding the mean vector. Because samples were used in the Fisher analysis, this cone is larger, but probably more indicative of the true scatter at the sampling locality than if all specimens had been weighted equally.

The direction of magnetization furnishes a Triassic north pole in eastern Siberia (Fig. 2, No. 1), as follows:

$$\text{Lat.} = 65^\circ\text{ N.}; \quad \text{Long.} = 156^\circ\text{ E.}; \quad \delta m = 15\tfrac{1}{2}^\circ; \quad \delta p = 14\tfrac{1}{2}^\circ,$$

with δm, δp, semi-axes of the 95 per cent confidence ellipse. Since our investigation began, results of work in the same general area have been published[3] (pole 2);

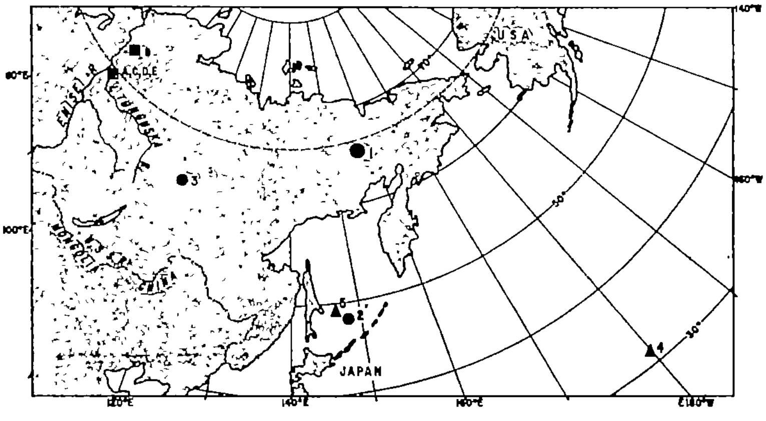

Fig. 2. Location of the north pole with respect to Siberia and Europe, from palæomagnetic measurements on Triassic rocks. Numbers refer to results quoted in this article (1), and by Makarova (ref. 3) (2), Fineberg and Dashkevich (ref. 4) (3), Khramov (ref. 5) (4), and Nairn (ref. 6) (5). Sampling sites are represented by letters

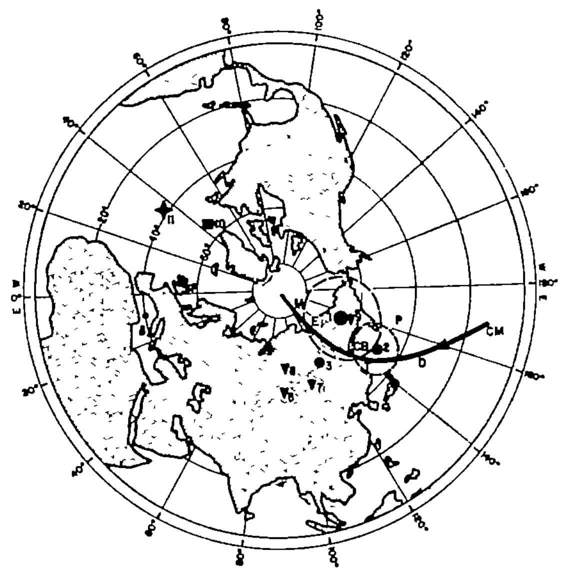

Fig. 3. Location of the North Pole, estimated from palæomagnetic measurements on Triassic rocks. Symbols represent polar plots with respect to: ●, Siberia; ▼, N. America; ■, Africa; ✦, Australia. Numbers indicate source material: 1, our results; 2, 3, other work on Siberian traps (refs. 3, 4); 6, Connecticut lavas (ref. 7); 7, Springdale Sandstone (ref. 8); 8, Brunswickian sediments (ref. 7); 9, New Oxford sediments (ref. 9); 10, Bechuanaland Cave sandstone (ref. 10); 11, Brisbane tuff (ref. 11). The solid line is a polar path relative to Europe, typical of versions published by various authors. *CM*, Cambrian; *D*, Devonian; *CB*, Carboniferous; *P*, Permian; *T*, Triassic; *E*, Eocene; *M*, Miocene

a report[4] on the magnetization of trap rock from the Angara River basin is the source for pole 3.

Palæomagnetic information unlocked from Triassic sedimentary exposures in Europe has yielded pole 4 relative to Russia[5], and pole 5, which is an average quoted by Nairn[6], of British, German and French data by different authors. Our pole, though located rather far north, conforms within limits of error to some point on the smoothed 'European' pole track (Fig. 3). This is not surprising, for it seems likely that in executing any movements inferred from this curve, Europe and northern Asia would have acted as one unit; hence, barring major changes in the Earth's radius, any two contemporary poles from Europe and Siberia should coincide.

Our result differs from three of four pole positions relating to North America[7-9] and, more drastically, from the African[10] and Australian[11] poles. Comparison of global non-Triassic data has revealed many similar discrepancies: the separation of the European and Indian curves exceeds 90° in the Jurassic, the earliest period for which Indian data exist[12]. One can interpret this by invoking large relative displacements between the two land masses, and probably by implication, between India and northern Asia. However, a direct comparison with the Siberian results must await examination of suitable Triassic exposures in India.

We are indebted to Prof. V. V. Fedinsky of the Physics of the Earth Institute, Moscow, and Dr. N. I. Marochkin, director of the Union Geological Research Institute, Leningrad, for making available the rock collection on which this work depended. At the University of Alberta, particular thanks are due to Dr. G. D. Garland, professor of geophysics, for providing workshop facilities and instrumentation, and for his continued interest.

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GEOLOGICAL RECONNAISSANCE IN VICTORIA LAND, ANTARCTICA

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IN the summers of 1957–58, 1958–59 and 1959–60, three small expeditions from the Victoria University of Wellington investigated approximately 2,500 sq. m. of ice-free terrain west of McMurdo Sound in southern Victoria Land, Antarctica. The first two expeditions spent a total of 80 days in the field, carrying out topographic surveying, meteorological[1] and gravity studies[2], palæomagnetic sampling[3], zoological and botanical studies, and geological surveys. This article summarizes the results of geological surveys made during the first two expeditions and cites references to papers awaiting publication.

Physiography

The inland ice plateau of eastern Antarctica is bounded at approximately longitude 160° E. by a coastal mountain chain extending 1,000 miles from latitude 70° S. to 85° S. North of latitude 80° S., this mountain chain lies within Victoria Land. Nearly all Victoria Land is completely glacierized; major outlet glaciers flow from the inland ice plateau through the coastal mountain chain to the Ross Sea.

The deglacierized terrain investigated comprises about 2,500 sq. miles of lowland valleys and mountain-

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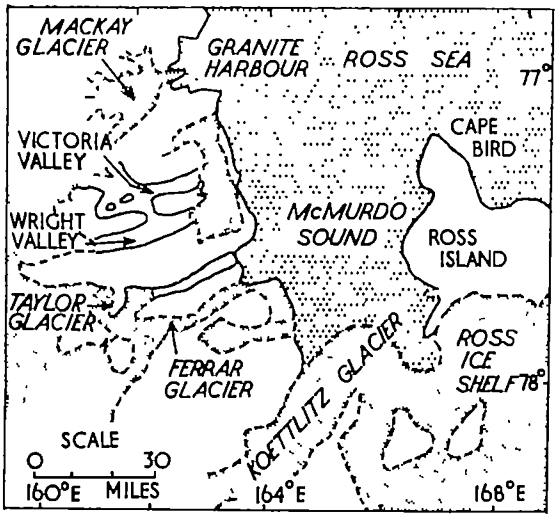

Fig. 1. Locality map of McMurdo Sound region

ous divides lying between the Mackay Glacier (77° S.) and the Taylor and Ferrar Glaciers (77° 45′ S.) (Fig. 1). The Wright Valley and the Victoria Valley system were formerly occupied by glaciers flowing eastwards from the inland ice plateau to McMurdo Sound.

General Geology

In this area, a peneplained Precambrian or lower Palæozoic crystalline basement complex is unconformably overlain by more than 4,000 ft. of Beacon Group sediments (Table 1). Sills and dykes of tholeiitic dolerite intrude both basement complex and Beacon sediments[4].

The Basement Complex. The basement complex exposed in the Wright Valley and Victoria Valley system comprises more than 15,000 ft. of highly folded Precambrian marbles, hornfelses and schists, intruded by acid orthogneiss and coarse porphyritic biotite-hornblende granite. The acid orthogneiss occurs in belts separating the meta-sediments from the biotite-hornblende granite. The meta-sediments strike approximately north to north-west. The foliation of the orthogneiss and porphyritic biotite-hornblende granite also trends north to north-west, parallel to the strike of the meta-sediments. Numerous intrusions of pegmatite and granodiorite dykes with no regular orientation cut through the orthogneiss and the porphyritic biotite-hornblende granite. In the eastern Wright Valley, a swarm of microdiorite dykes dipping south-west intrudes orthogneiss near its contact with the meta-sediments.

Table 1. GENERALIZED GEOLOGICAL HISTORY FOR WRIGHT VALLEY–VICTORIA VALLEY SYSTEM, VICTORIA LAND

Age	Description
Quaternary	At least four phases of glacial cutting and deposition of moraine.
Quaternary to ? Upper Tertiary	Eruption of kenyte, basalt, phonolite and trachyte lavas around McMurdo Sound. Small basaltic cones formed in Taylor Dry Valley, but not in Wright and Victoria Valleys.
? Mesozoic	Intrusion of Ferrar dolerite sills and dykes into the basement complex and overlying Beacon Group sediments.
Mid-Mesozoic to Mid-Palæozoic	Deposition of Beacon Group sediments, including sub-greywacke, arkose orthoquartzite, siltstone and carbonaceous shale facies.
	Major unconformity, with peneplanation.
Lower Palæozoic to Precambrian	Tightly folded, (?) Precambrian marbles, hornfelses and schists intruded by acid orthogneiss and younger granite, granodiorite and diorite. Profuse intrusions of younger acid and basic dyke swarms.

An extensive body of homogeneous biotite-hornblende granite ('pink granite' of early workers) intrudes and in many localities overlies the meta-sediments, gneisses and porphyritic granites. Exposures of hornblende-plagioclase amphibolite up to 500 ft. across occur within this younger granite.

Dense swarms of north-east striking lamprophyre and porphyry dykes intrude all basement rocks and in this region are the youngest intrusives of the basement complex.

Beacon Group Sediments. More than 4,000 ft. of Beacon Group (Mid-Palæozoic to Mid-Mesozoic in age) sediments are exposed in the western part of the Taylor and Wright Valleys and the Victoria Valley system, resting unconformably on the peneplained basement surface[5].

These sediments were examined in detail throughout an area of 800 sq. miles, and are subdivided into five lithofacies. From the base of the group the upward succession is :

(1) Sub-greywacke facies : up to 300 ft. of dark, well-indurated conglomerates, sandstones and siltstones. Bedding structures include thin graded beds, cross-beds, and small-scale slump structures.

(2) Arkose facies : at least 500 ft. thick and overlying the sub-greywacke facies, or more commonly resting on the peneplained surface of the basement complex. Bedding structures include small-scale cross-bedding and thick sequences of black, green, red and white beds repeated rhythmically. Ferruginous concretions are abundant in this facies.

(3) Orthoquartzite facies : at least 2,500 ft. thick and characterized by both massive and large-scale cross-bedded units, with thin interbedded lenses of arkose, subarkose and siltstone.

(4) Red-green siltstone facies : varying between 70 and 300 ft. in thickness and observed only on the southern side of Taylor Glacier, where it overlies the orthoquartzite facies. The sediments comprise thin alternating red and green siltstone units interbedded with beds of arkose and orthoquartzite. Mud-crack horizons and concentrations of worm borings are common in this facies.

(5) Carbonaceous shale facies : at least 500 ft. thick and comprising thin, impure coal seams alternating with cross-bedded and slumped sandstones and siltstones. This facies is well exposed near Pyramid Mountain on the southern side of Taylor Glacier, these rocks being the youngest Beacon Group sediments examined during this investigation.

Ferrar Dolerites. The Ferrar Dolerite sills examined have a total thickness of at least 3,000 ft., distributed through a vertical range of 7,000 ft. (ref. 6).

The lowest sill observed is an undulating sheet up to 800 ft. thick, usually intruded into the granites of the basement complex. However, where this sill encounters steeply dipping meta-sediments, it rises along the bedding planes to the peneplained basement surface and intrudes diagonally up into Beacon Group sediments, thinning markedly as it does so.

A younger sill, approximately 700 ft. thick, has penetrated the unconformity between the basement rocks and the overlying Beacon sediments, and in some places intrudes up to 500 ft. above the peneplain surface. Rarely it intrudes granites below the peneplain surface.

Few of the sills intruding Beacon sediments exceed 400 ft. in thickness. Their pattern of emplacement is extremely irregular and they frequently divide into thinner units. Large feeder dykes have been observed to rise to these sills.

The lowest two sills are mineralogically similar and display marked variation. In their lower levels they are composed essentially of hypersthene, augite and calcic plagioclase, whereas their upper levels consist of augite, pigeonite, less calcic plagioclase, and considerable amounts of micrographically intergrown quartz and alkali feldspar. Autoliths of pyroxene aggregations, up to 2 ft. in diameter, and feldspathic veins occur in the lower levels. The upper levels are characterized by pegmatitic veins and schlieren, and show considerable deuteric alteration.

The sills which intrude Beacon Group sediments are relatively homogeneous, consisting of augite, calcic plagioclase and abundant cryptocrystalline mesostasis.

Quaternary Glaciation. Evidence of four glaciations is recognized in the Wright Valley and Victoria Valleys system[7]. The first two were the most intense. Both carried ice from the inland plateau to McMurdo Sound and the Ross Sea. Highly weathered moraines of the first glaciation lie on glacial benches at heights of 4,500 ft. above sea-level. The second glaciation is represented by a thin veneer of highly weathered moraine on the floors of the present valleys and bench remnants up to 2,000 ft. above sea-level.

Thick moraines of a third glaciation partly overlie the second glaciation moraines, indicating a less-extensive advance into the valleys by plateau, alpine and coastal piedmont glaciers. Associated with the moraines of the third glaciation are salt lakes, evaporite deposits, high-level lake shorelines, dune sands and extensive areas of desert pavement strewn with ventifacts.

The limited distribution of thick coarse moraines in the western part of this area indicates that during a fourth glaciation the remnants of the valley glaciers fed from the plateau advanced only short distances eastwards towards the ice-free central portion of the valleys.

Each glaciation resulted from a build-up in the surface elevation of the plateau, allowing ice to spill over high rock thresholds at the valley heads. With each succeeding glaciation, net increases in elevation of the plateau diminished, until during the third and fourth glaciations it was sufficient to allow only a small volume of ice to flow into the valleys.

Since the fourth glaciation, the volume of ice flowing over the valley-head threshold has been negligible. Plateau, alpine and piedmont glaciers are in the final stages of recession from this area.

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SPECTRAL CHARACTERISTICS OF AGGREGATED CHLOROPHYLL AND ITS POSSIBLE ROLE IN PHOTOSYNTHESIS

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IN 1951 Duysens[1] and French and Young[2] reported the fluorescence (720 mμ) of an unknown pigment in the red alga *Porphyridium cruentum*. The fluorescence was attributed to chlorophyll *d* (ref. 1), which had been discovered by Strain[3] in other algae of this group. Because it absorbed at longer wave-lengths than chlorophyll *a*, and because of the highly efficient transfer of energy to it, Duysens assigned to this pigment the role of an energy trap to explain the low yield of chlorophyll-sensitized photosynthesis—especially on the long wave-length side of the red chlorophyll band. However, it was later determined that there is no chlorophyll *d* present in *Porphyridium*[4].

Recently[5], an emission band at 720 mμ was reported for chlorophyll *a*, both *in vitro* and *in vivo*, and was attributed to an aggregated form of chlorophyll. The present investigation shows that the aggregate is a dimer.

The absorption spectrum of the dimeric form of chlorophyll in ethanol was determined (at 293° K.) from the difference in absorption between dilute (10^{-5} *M*) and concentrated ($>10^{-2}$ *M*) solutions of chlorophyll.

Production of the aggregate is accompanied by a symmetrical splitting of monomer bands which is characteristic[6] of dimer formation. The first excited state of the monomer, 665 mμ, gives rise to absorption maxima at 682 and 648 mμ, with absorption coefficients ε_2 and ε_1, respectively (Fig. 1). One would also expect splitting of the second excited state; however, only one absorption band, at 450 mμ, was resolved. The minor absorption bands also appear to split. From its spectroscopic properties, it was deduced that the dimer has the geometry shown in Fig. 2. By using the relationship $\frac{\varepsilon_1}{\varepsilon_2} = \tan\left(\frac{\pi - \theta}{2}\right)$, the angle θ was found to be 130°.

The source of the asymmetry of the long wave-length band (683 mμ) in Fig. 1 may be a metastable

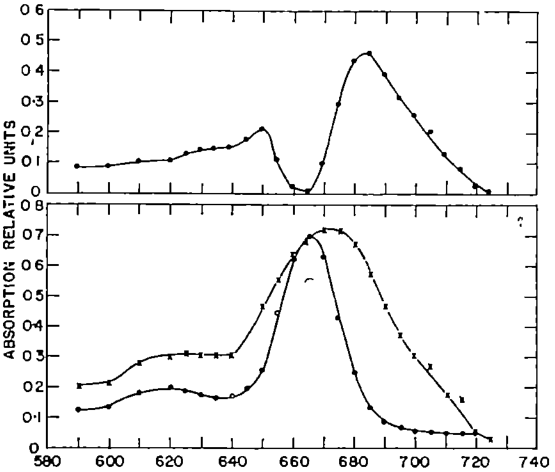

Fig. 1. The curves in the lower graph show the absorption of 10^{-5} M (—o—o—) and 3×10^{-3} M (—×—×—) solutions of chlorophyll *a* in ethanol. The difference in absorption between these two curves is plotted in the upper graph. This difference spectrum, with maxima at 682 and 648 mμ, is considered to be the absorption spectrum of the dimeric form of chlorophyll at long wave-lengths

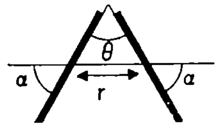

Fig. 2. Geometry of the dimeric form of chlorophyll *a*. Heavy lines depict the planes of the molecules; the angle $\theta \simeq 130°$, and r (the distance between the centres of charge density) $\simeq$ 12 Å.

transition, with absorption maximum at about 705 mμ. Emission from the dimer, at 720 mμ, may originate from this state.

The equilibrium constant for the reaction $2\ \text{Chl}_M \rightleftharpoons \text{Chl}_D$, in ethanol, was found to be approximately 30 mole^{-1}, where Chl_M and Chl_D are monomeric and dimeric chlorophyll molecules, respectively. Observations of the concentration of the monomeric form in the temperature-range 293°–77° K. show that the equilibrium between these two forms is almost independent of temperature. On the basis of the precision of the measurements, the heat of the reaction, ΔH, is estimated to be less than 20 cal./mole. It was assumed that there are two pathways not dependent on temperature and one pathway dependent on temperature by which the excitation energy of the dimer may be dissipated. The pathways independent of temperature are emission and internal conversion; the pathway dependent on temperature is collisional deactivation (*in vivo*, energy-storing chemical reactions will predominate over collisional losses). The rate constant, k_3, for the deactivation pathway dependent on temperature was equated to $A = \exp(-\Delta E/RT)$. In this expression, ΔE is the activation energy and A is the Arrhenius constant. Values of ΔE and A were determined in the following manner. An expression was derived for yield of emission φ from the dimer as a function of temperature:

$$\ln\left(\frac{1}{\varphi} - \frac{1}{\varphi 77°\ \text{K.}}\right) + \ln\frac{k_1}{A} = \frac{-\Delta E}{RT}$$

where φ77° K. is the yield of emission at 77° K. (found to be 0·8 *in vitro* and 0·1 *in vivo*) and k_1, the reciprocal of the emission life-time, is the rate-constant for the emission process ($k_1 = 1/10^{-4}$ sec.). A plot of the above equation gave: $\Delta E = 1{\cdot}0$ k.cal./mole *in vivo* and 0·9 k.cal./mole *in vitro*, and $A \approx 10^5$ sec.$^{-1}$.

This value of A for the dimer is considerably lower than is usually found for uni- or bi-molecular reactions, and may be considered a measure of the *low* transition probability from the excited triplet state to the singlet ground-state.

A relationship was derived between the ratio of dimer to monomer emission (at 77° K.) and the total concentration of chlorophyll. This expression was in good agreement with the experimental results. Included in the derivation is the assumption that a dimeric form of chlorophyll gives rise to emission at 720 mμ. This mathematical relationship was plotted in graphical form, from which it was possible to estimate the concentration of monomer and dimer in *Porphyridium*, *Chlorella*, *Polyedriella* and *Muriella* to be: 3·2 and 2·0, 1·9 and 0·8, 1·6 and 0·6, and 1·0 and 0·2 × 10^{-3} M, respectively. These organisms are arranged in order of decreasing dimer/monomer concentration; it is significant that the wave-number at which the photosynthetic decline occurs decreases along the series.

Since a number of emission measurements were made at low temperatures, it was thought necessary to determine the effect of freezing the cells to 77° K. and then thawing them.

An electron microscope study of material treated in this manner showed a small amount of shrinkage and formation of vacuoles, but no real evidence of disruption of chloroplasts.

Chlorella cells were frozen to 77° K., thawed, and then examined for the Hill reaction. Their ability to carry out this photochemical step (even if with diminished yield) and the fact that changes in absorption were not noted after freezing and thawing, were taken as indicating that these processes introduce no gross changes in the photosynthetic apparatus, or in concentration or aggregation of pigment. It might be added that snap freezing is a method of preservation of activity of the Hill reaction[7].

The action spectrum for exciting emission from the dimer was measured *in vitro* at 77° K., and *in vivo* in several different photosynthetic organisms, at 77° K. and 293° K. The results are in qualitative agreement with the absorption spectrum of dimer in solution.

Action spectra measurements with *Porphyridium cruentum* were made at 77° K. to determine the contribution of the various pigments to emission by chlorophyll. These indicate that light energy absorbed by phycoerythrin is transferred to both monomer and dimer; however, more is transferred to the monomer. Energy absorbed by phycocyanin or the chlorophyll monomer favours transfer to the dimer.

The difference between the action spectrum for excitation of fluorescence at 685 mμ and that at 720 mμ supports the contention that there is more than one form of chlorophyll *in vivo*. A review of the evidence, both ours and other workers[8-10], favouring the existence of two (or more) forms of chlorophyll *in vivo*, will be given in detailed papers which are in preparation.

The concept of aggregated chlorophyll has been used to interpret concentration quenching in solution[11,12]. Lavorel[11] has suggested that the long wave-length decline in fluorescence and photosynthesis might be explained by the presence of an inactive chlorophyll dimer *in vivo*.

We conceive of the dimer as being required for participation in photosynthesis, and propose as necessary in this process the interaction of two unstable products produced by light, one (UP_M) formed by the monomer, and the other (UP_D) by the dimer, respectively. The first stable product, S, of photosynthesis is formed by the dark reaction between UP_M and UP_D. These steps are summarized by the following reactions:

$$X + \mathrm{Chl}_M{}^* \rightarrow UP_M + \mathrm{Chl}_M$$
$$Y + \mathrm{Chl}_D{}^* \rightarrow UP_D + \mathrm{Chl}_D$$
$$UP_M + UP_D \rightarrow S$$

The unstable products may have life-times of the order of a minute and may be identical with the light-induced radicals reported *in vivo* by Commoner *et al.*[13] and Sogo *et al.*[14].

On the basis of the existence of a monomeric and dimeric form of chlorophyll *in vivo*, and the assumptions made above, we will try to give a relatively simple explanation and indicate relationships for a number of the observations in photosynthesis.

It is postulated that some monomer molecules are associated with dimer molecules; direct absorption by these monomer molecules results in energy being transferred to dimer molecules—so that absorption by the monomer leads to formation of both unstable products. However, when the dimer alone is absorbing light, the yield of emission is low and UP_M is not formed. This is the situation that occurs when photosynthesis or fluorescence is sensitized by light absorbed on the long wave-length side of the chlorophyll maximum. Addition of steady or intermittent light of shorter wave-length to that at longer wave-length will permit the reaction sensitized by the monomer to proceed, resulting in full photosynthetic efficiency. As shown above, the onset of the long wave-length decline is related to the concentration of dimer found in various organisms.

Increase in fluorescence yield with light intensity[15] could result from decomposition of the dimer to monomer.

Changes in absorption induced by light[10] may result from shifts of monomer-dimer concentration, or from changes in position of dimer absorption maxima (determined by the angle θ—which, in turn, appears to be effected by such factors as hydrogen ion concentration).

Details of this work will be published elsewhere. We thank Prof. H. Linschitz, of Brandeis University, for his co-operation and provision of apparatus. Part of this work was done at the Air Force Research Center, Bedford, Mass. This work was supported in part by a grant from the National Science Foundation to Brandeis University (grant *G*–6418).

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POLYMER MONOLAYERS

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IN a recent communication[1] Ries, Beredjick and Gabor described the surface behaviour of polyvinyl benzoate, which showed several unusual features, and directed attention to the wide variation of polymer surface properties with molecular structure. We have recently studied monolayers of three sets of polymers of different structural types, and the results further accentuate the complexity of behaviour obtainable with monolayers of vinyl-type polymers.

The first set of polymers was a series of samples of polymethyl acrylate the viscosity-averaged molecular weights of which varied from $2{\cdot}6 \times 10^4$ to $4{\cdot}75 \times 10^6$. The second set of polymers were graft copolymers of *iso*butene and methyl acrylate in which the number and length of polymethyl acrylate (PMA) branches were varied on a fixed poly*iso*butene (PIB) backbone ('Vistanex' *LM/MS*, $\overline{M}_v$ $4{\cdot}5 \times 10^4$). The third set comprised a number of samples of chlorosulphonated PIB ('Vistanex' *LM/MH*, $\overline{M}_v$ $7{\cdot}2 \times 10^4$) with varying chlorine and sulphur contents, and their hydrolysis products in which the sulphonyl chloride groups had been substantially, if not wholly, converted to sulphonic acid groups. The graft copolymers were prepared by an established photochemical bromination-debromination technique[2] and the Reed-Horn reaction[3] was used for the chlorosulphonation of PIB. Film balance measurements were made at 25° C. on dilute acid (N/100 hydrochloric acid) and other substrates with benzene used principally as spreading solvent. A few results obtained with acid substrate are given in Table 1 and Fig. 1 which include also, for comparison, the results given by Ries, Beredjick and Gabor[1] for polyvinyl benzoate (PVB), polyvinyl acetate (PVAc) and stearic acid.

PMA was found to behave similarly to PVAc but was rather more compressible. The most interesting feature of the PMA results was the observed significant variation in limiting area (23·0–26·5 Å.^{2}/monomer unit) over the range of molecular weight studied. Previously some workers have claimed limiting area to be independent of molecular weight[4]. Others have

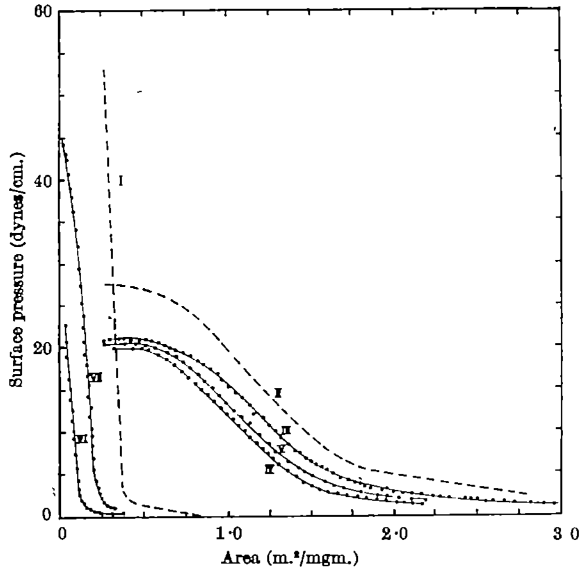

Fig. 1. Pressure-area isotherms. I, isotherm for polyvinyl benzoate (ref. 1); II, isotherm for polyvinyl acetate (ref. 1); III, isotherm for polymethyl acrylate, $\overline{M}_v$ 2·73 × 10^6; IV, isotherm for polymethyl acrylate, $\overline{M}_v$ 3 2 × 10^4; V, isotherm for PMA/PIB graft polymer; VI, isotherm for chlorosulphonated PIB; VII, isotherm for hydrolysed chlorosulphonated PIB

found some increase with molecular weight[5] but not as great as that reported here. However, it does seem that we have gone to rather higher molecular weights than most other workers. It is probable that, with flexible molecules, as the molecule-length increases, intermingling of molecules becomes increasingly restricted so that at the higher molecular weights packing on the surface becomes less efficient.

PMA and PVAc monolayers behave rather similarly, and the behaviour of PVB makes an interesting contrast. In the case of PVB the high collapse-pressure is attended by a low limiting area. The preferred explanation[1], in terms of the postulated formation of rigid coils or folds rising from the surface, would also account for the high resistance to compression in the linear region of the isotherm. A further interesting feature of the PVB isotherm is the very low resistance to compression at areas greater than 0·4 m.^{2}/mgm. In this region monomer units are being forced out of the surface to form the rigid overfilm; the feeble resistance to compression in this region indicates that this ejection from the surface is much more easily achieved with PVB than with PMA and PVAc which do not attain such a high collapse-pressure, but which reach their maximum resistance to compression at areas of approximately 1·5 m.^{2}/mgm.

Table 1. MONOLAYER PROPERTIES

	Limiting area		Collapse pressure (dynes/cm.)	Compressibility (cm./dyne)
	m.^{2}/mgm.	Å.^{2}/monomer unit		
Stearic acid*	0·43	20†	42	0 0019
Polyvinyl acetate*	1 90	27	25	0 0252
Polyvinyl benzoate*	0 38	9	53	0·0056
Polymethyl acrylate	1·61–1 86	23·0–26 5	21	0·080
PMA/PIB graft (1 02 : 1 w/w, 2–3 PMA branches per mol.)	1 68	23‡/10·7§	20 4	0·030
CS–PIB (S, 3 3 per cent, Cl, 10 per cent)	0·12	c. 1·5	28	0·030
HCS–PIB (S, 2·4 per cent; Cl, 7 8 per cent)	0 23	c. 3	44	0·015

* Ref. 1.
† Area per molecule.
‡ Assumed area per PMA monomer unit.
§ Calculated area per PIB unit.

Poly*iso*butene does not spread at all at the air-water interface, neither does brominated PIB at least up to 14·4 per cent bromine content. Mixtures of PMA and PIB were found to behave just the same as if the PIB were not present. This suggests that with the mixture the PIB exists on the surface in a completely collapsed state and its presence in no way affects the behaviour of PMA. It is assumed, therefore, that with the PMA/PIB graft copolymers the behaviour of the PMA branches is not appreciably affected by the presence of the PIB backbone and that the limiting area of the monomer units in the PMA branches is close to that found for homopolymer of similar degree of polymerization, although the two limiting areas may not be identical due to some restriction of the packing of PMA when present as branches. On the basis of this assumption, it was immediately obvious from the results obtained with the graft copolymers that the PMA branches fell short of accounting for the total observed limiting area and that some 11 Å.^{2} must be occupied per *iso*butene unit in the case of the graft copolymer cited in Table 1, which had some two to three branches of PMA ($\overline{P}_v$ 3·21 × 10^2) grafted on to the PIB ($\overline{P}_v$ 8·04 × 10^3). Models of the *iso*butene unit projected on to a plane surface give areas ranging from 14 Å.^{2} to 22 Å.^{2} for various orientations. While the accuracy of calculating in this way the area occupied by an *iso*butene unit in the graft copolymer is debatable, the value obtained (10·7 Å.^{2}) does indicate a surprisingly large extension of the backbone by a few horizontally orientated polymeric branches. Indeed, the result suggests that the actual number of branches present considerably exceeds the number calculated (2–3) from PMA content and average degree of polymerization. The branch degree of polymerization was assumed to be the same as the $\overline{P}_v$ of a PMA homopolymer prepared under similar conditions of rate, solvent (cyclohexane) and transfer agent (triethylamine). This method of calculating branch frequency overlooks, by its very nature, the possible existence of numerous branches much shorter than the average.

Hydrolysed chlorosulphonated PIB polymers (HCS–PIB) gave high collapse-pressures, up to 44 dynes/cm. The unhydrolysed chlorosulphonated polymers (CS–PIB) gave much lower collapse-pressures and were much less resistant to compression than their hydrolysis products. Calculation of limiting areas for these randomly substituted polymers in terms of Å.^{2} per monomer unit are rather approximate. The values thus obtained are very low and indicate, as one would expect from the relatively infrequent occurrence of polar groups, that these polymers are not horizontally orientated. Areas occupied per polar group were in the range 20–30 Å.^{2}, that is, comparable with the limiting area per molecule of stearic acid. It is evident that the hydrocarbon chain forms loops above the surface and this permits fairly close packing of polar groups. In spite of some similarity with stearic acid type behaviour, these polymer films are much more compressible: more compressible in fact than PVB (horizontally orientated, high collapse-pressure) and more akin, in this respect, to PVAc and PMA (horizontally orientated, low collapse pressure).

Less highly substituted chlorosulphonation derivatives of PIB were, as expected, less surface-active than the examples given in Table 1. More highly substituted derivatives were also less active ; this is attributed to partial solubility since with increasing substitution there is an increasing possibility of highly polar groups occurring sufficiently close together to render portions of the molecules soluble in water.

HCS–PIB polymers gave solid films, while the CS–PIB, PMA and graft polymer films were fluid.

A detailed account of this work and further discussion is being prepared for publication.

Acknowledgment is made to the Chief Scientist, Department of Supply, South Australia, for permission to publish this work.

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HIGH-YIELD CRYSTALLIZATION OF UREASE FROM JACK BEAN

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CRYSTALLIZED urease of jack bean was obtained by Sumner[1] by quite a simple procedure, but very few samples of jack bean are suitable as the starting material for this method. The causes of the difficulties are believed due to : (1) the samples contained a relatively small amount of urease[2] (less than 90 units per gm.) ; (2) in the case of some samples urease is difficult to extract with 32 per cent acetone, as the first step of method, while containing the usual amount of enzyme[3]. Jack beans cultured in Japan belong to this type[4].

To obtain the preparation of urease crystals from these samples is impossible by Sumner's method. The present investigation was undertaken to find a method of purification of urease which is applicable to such samples.

In an attempt to provide crystallized urease by the application of methods usual for other enzymes it was observed that, as the purification proceeds, urease was apt to be converted so as to be insoluble in water, especially after treatment with acetone. This insoluble form of urease with its activity still preserved has already been studied by Sumner[5]. Because of this, any kind of usual fractionation procedure was not applicable for further purification. This insoluble form was changed into a soluble one by mixing with urea ; but after dialysation it was not susceptible to any fractionation, and SH_2 seemed to have little ability to dissolve this insoluble form. Uehara and Kobashi demonstrated that cysteine can convert this insoluble form into the soluble form[6]. I used 2-mercaptoethanol instead of cysteine and devised a method of preparation of crystalline urease with good yield from jack bean, though it contains a low concentration in units of urease.

Jack beans cultured in Japan, *Canavalia ensiformis* var. *gladiata*, were used. They were pulverized into fine powder which contained 100–180 units of urease per gm. The defatting treatment was unnecessary for the present purpose. Acetone was treated with a small amount of potassium permanganate and anhydrous potassium carbonate, then distilled. Calcium phosphate gel was prepared by the method of Tsuboi and Hudson[7].

Both the colorimetric timing method[8] and Iwasaki's azotometry[9] were used for the assay of urease activity under conditions devised by Sumner and Hand[2], and the latter was employed when an accurate value was required. For the determination of protein nitrogen the sample was first digested as usual and the nitrogen of ammonium salt was determined by Iwasaki's azotometry[10]. Protein including urease[11] was calculated by assuming that it contains 16 per cent of nitrogen. The specific activity, that is, units per gm. of protein, was calculated from the foregoing data.

Utilization of several procedures usual in the purification of other enzymes has been studied, of which the following series of procedures was most satisfactory. All work was carried out at room temperature, and distilled water made by a gilded-tin vessel was used throughout the work. 500 gm. of jack bean meal to which 2·5 litres of water had been added and stirred for 10 min. was first filtered through a cloth sack. The residue was again treated with 1·0 litre of water and treated in the same manner, then both filtrates were collected, suspended together with 'Celite' and filtered again through a Büchner funnel. About 3 litres of yellowish brown, slightly turbid, filtrate was obtained.

To 3 litres of filtrate, 1,370 gm. of solid ammonium sulphate was added to give 60 per cent saturation. After a few hours, the resulting precipitate was collected by centrifugation and dissolved in 700 ml. of water. The solution was dialysed against a stream of tap water for about 24 hr., enough time for there to be precipitated the considerable amount of resulting denatured impurities ; these were centrifuged off and discarded. Approximately 1,700 ml. of dialysate was obtained.

The dialysate was mixed with 850-ml. portions of calcium phosphate gel. The *p*H of the mixture was adjusted to 5·0–5·5 with 1 *N* acetic acid and allowed to stand for 20 min. with gentle stirring. The gel was collected by centrifugation. It has been shown from experimental results that for each 100 mgm. of protein in the dialysate 50 mgm. of calcium phosphate gel in dry weight was necessary for almost complete adsorption of urease. Usually 1 ml. of dialysate includes 13–18 mgm. protein, and the same amount of gel includes 14 mgm. of calcium phosphate; accordingly, one might use expediently one volume of gel to two volumes of dialysate for adsorption of the enzyme.

Elution was accomplished by gentle mixing of the gel, avoiding foaming with 200 ml. portion of $M/15$ phosphate buffer, *p*H 7·5, for 15 min. This is done three times, and the eluates obtained after centrifugation in each case were pooled. Total eluate was approximately 600 ml. and displayed the activity of between 6,000 and 8,000 units per gm.

The eluate was made up to 50 per cent saturation with ammonium sulphate by adding 230 gm. of it and kept for 5 hr. At the end of this time, the precipitate was recovered by relatively high-speed centrifugation, dissolved in 80 ml. of water and the enzyme solution was dialysed against 2 litres of distilled water for 15 hr. (or overnight) in the cold. The outer fluid was renewed three times during the dialysis and about 150 ml. of dialysate was obtained, of which activity was about 12,000 units per gm.

Urease in the dialysate was changed into insoluble form by precipitation with acetone. At first the dialysate was brought to $M/15$ referring to potassium phosphate by addition of either solid potassium dihydrogen phosphate or 0·5 M of its solution. If some amount of precipitate had appeared during the period of dialysis it was usually redissolved by this treatment. (This precipitate contains considerable urease so it should not be discarded.) Urease in solute state was then precipitated by dropwise addition of 0·6 vol. of acetone with continuous stirring. To ensure that the urease is converted completely into insoluble form, the mixture was allowed to stand for 2 hr. at room temperature. Then the precipitate containing the insoluble form of urease was collected by centrifugation and washed with a small portion of water. Usually 93–99 per cent of urease activity in dialysate was recovered in the precipitate by this procedure.

The insoluble urease is made soluble by the action of 2-mercaptoethanol and the majority of the impure protein rejected as the insoluble part, so that the specific activity will increase considerably in this step.

A mixed solution composed of 36 ml. of 0·025 M citrate buffer *p*H 7·0 and 4·0 ml. of ten times diluted aqueous solution of 2-mercaptoethanol was added to the acetone precipitate, stirred enough to be homogeneous and allowed to stand for 1·5 hr. with intermittent stirring, after which the remaining precipitate was centrifuged off. In this instance, approximately 70 per cent of the activity was transferred to the supernatant, amounting to 40 ml. After this was adjusted to *p*H 6·0 with 0·1 M citric acid, 0·3 vol. of acetone (12 ml.) was added and kept in an ice chamber at 5° C. Urease was soon crystallized out and the crystallization was completed within a few hours.

The crude crystals generally included a small amount of insoluble impurities. The recrystallization was easily achieved by the method of Dounce[12]. The content of nitrogen, urease units, specific activity and yields at each step in each instance are shown in Table 1, as an example.

Urease was purified some 1,000 fold from starting material with yield 23–33 per cent and crystals were also obtained from material showing a low activity of only 65 units per gm. Attempts to simplify the procedure described here were made, but a satisfactory result was not obtained.

Table 1

	Total N (mgm.)	Total units (×100)	Specific activity (units/gm.)	Yield (per cent)
Starting material	—	680	136	100
Water extract	23,500	630	428	92·5
First ammonium sulphate fractionation	5,290	575	1,740	84·4
Calcium phosphate gel eluate	885	378	6,830	55·5
Second ammonium sulphate fractionation	458	326	11,400	47·9
Precipitate with acetone	378	300	12,700	44·1
Redissolved solution	95·2	236	39,700	34·7
Crude crystal	41·4	221	85,400	32·5
Recrystallization once	29·7	212	114,000	31·2
Recrystallization four times	20·4	166	130,000	24·4

The urease crystals obtained in this way were colourless octahedrals in form of 3–8μ in diameter. A photograph by phase contrast microscope is presented in Fig. 1. The specific activity of pure urease crystals which were recrystallized four times was determined; they had an activity of 130,000 units per gm. The electrophoretic experiments with them were carried out in veronal buffer of ionic strength 0·05, *p*H 8·6 and in $M/30$ phosphate buffer, *p*H 7·0, respectively. Only one single and symmetrical peak was observed under these conditions.

Ureases which occur in some kinds of jack beans are with difficulty extracted with aqueous acetone, though these samples include sufficient amounts of urease in them[3,4]. They are unsuitable as starting material for the method by Sumner. However, almost all the urease in any sample is easily extracted with water; because of this the aqueous extract of urease was made in the first step. It is reasonable to assume that some obstructive factors may exist in these samples when aqueous acetone was used for the extraction of urease. Sumner demonstrated previously that purified urease was converted into insoluble form by the procedures consisting in adding ethanol and sodium chloride to its solution and keeping it at room temperature[5]; moreover, even crystalline urease gave the same result[13]. I found that, as the purification of urease

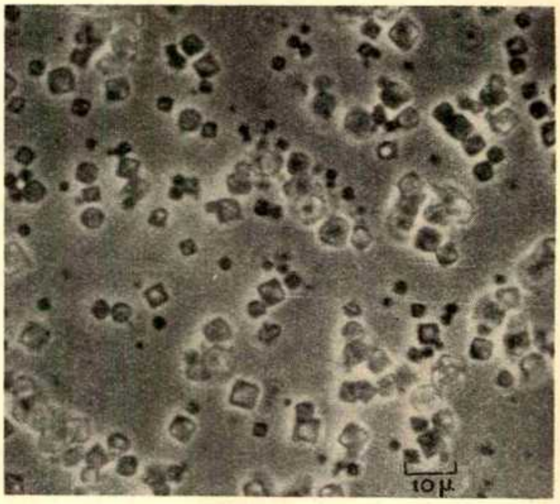

Fig. 1

proceeded, more than 10,000 units per gm. could be easily converted into the insoluble form by addition of acetone. Uehara and Kobashi proved that insoluble urease recovered its solubility by the action of cysteine or glutathione. In the present work, loss of activity was scarcely recognized when urease was converted into insoluble form by treatment with acetone. It recovered its solubility by the action of 2-mercaptoethanol ; at the same time a small portion of impure protein was also redissolved out of the insoluble precipitate. This was found as insoluble impurity when urease was recrystallized. From these points of view it is reasonable to infer that among the sulphydryl groups of the urease molecule there must be a special part which is relevant to the formation of the insoluble form as well as indifferent to the revealing of enzyme activity, and the formation of disulphide linkages does not occur only between urease molecules themselves but also between different protein molecules. Urease was crystallized with good yield starting from aqueous extract of jack bean meal ; whereas it might be unsuitable for Sumner's method. Urease crystals thus obtained were identical with respect to the crystal form and activity to those obtained by Sumner's method[14].

I wish to express thanks to Prof. K. Sekita for his kind guidance and constant encouragement throughout this work. Thanks are also due to Dr. K. Kobashi for his helpful suggestions. This work was supported in part by a research grant from the Taito-Pfeizer Co., Ltd.

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INHIBITION OF THE MULTIPLICATION OF INFLUENZA VIRUS BY ANILINE DERIVATIVES OF THE FRANCIS INHIBITOR

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It is generally accepted that the red-cell receptor concerned in hæmagglutination by myxoviruses is an analogue of the receptor substances of cells which are susceptible to infection[1]. Two findings seem to support the active role of the receptor[2] in virus multiplication : (1) its amount is subjected to a cyclic fluctuation during the process of multiplication[3] ; (2) both the early and late stages of multiplication are inhibited by the same chemical compounds[4].

To investigate this question, human serum α-inhibitor (Francis inhibitor[5]) was prepared from Cohn's 4/4 fraction as recommended by Hirst[6]. The inhibitor was completely inactivated at room temperature by any of the active influenza virus preparations tested.

The interaction between influenza virus A^1 Budapest 4/49 (*Bp*) and α-inhibitor is shown in Fig. 1,*A*. Equal volumes of virus and α-inhibitor solution were kept at room temperature. At intervals, 1 ml. samples were mixed with 0·2 ml. of a 5 per cent suspension of fowl erythrocytes, kept in an ice-bath for 15 min. and centrifuged. The erythrocyte sediment was taken up in 0·5 ml. phosphate buffer, incubated at 37° C. for 2 hr., and centrifuged. The supernatants were titrated by Takátsy's micromethod[7] for hæmagglutinin, to determine the amount of virus recovered from the erythrocytes. There was a sudden drop in the recoverable virus in the early minutes, followed, even if virus had completely disappeared, by a rise nearly up to the initial hæmagglutination titre.

The disappearance and reappearance of virus might be explained by a competitive antagonism between α-inhibitor and erythrocyte receptor. At the beginning, α-inhibitor, being in excess, prevents the adsorption of the virus on the erythrocytes, but later, as the virus gradually inactivates the inhibitor, more and more of it will become demonstrable.

According to Wolley's concept[8], a hæmagglutination inhibitor is expected to inhibit virus multiplication only if it combines with the virus more firmly than does the cell receptor. Mandel and Racker[9] found such an inhibitor to the *GDVII* virus in the intestinal tract of the mouse.

In attempting to prepare influenza virus inhibitors meeting this requirement, we coupled aniline derivatives by diazonium linkage to a purified preparation of α-inhibitor. Coupling was followed by a second purification consisting of fractionations with alcohol, and dialysis at 4° C. against a *p*H 7·2 buffer solution for 3 days. The purified preparation was stored in a frozen or lyophilized state. Control preparation (No. 17, Table 1) was submitted to all the procedures of coupling (mainly oxidative procedures) except for adding aniline derivative.

Table 1. RELATIVE INHIBITORY ACTIVITY OF MODIFIED α-INHIBITORS

Preparation No.	Modifying compound	D_{75} in hæmagglutination inhibitor units as tested with 10^4 ID_{50} of			
		PR8	*Bp*	Singapore 1/57 non-avid	Lee
3	*p*-Ethyl-aniline	7·0	0·8	11·6	0·75
8	Diethyl-*p*-phenylenediamine	7·6	3·2	10·2	3·0
1	*o*-Methyl-aniline	10·2	4·8	5·5	4·0
14	*o*-Azotoluene-diazonium chloride	225	10·0	100	10·0
17	None	204	204	> 204	110

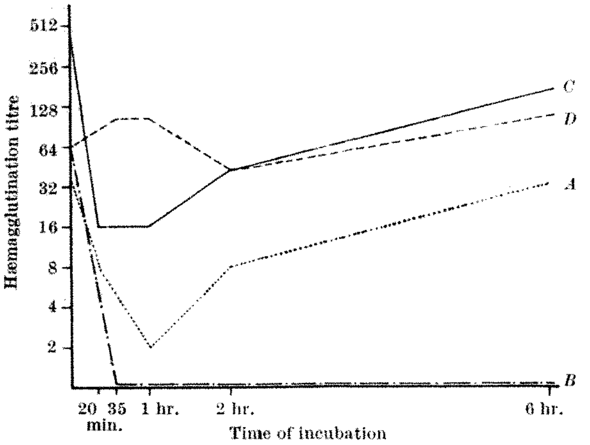

Fig. 1. Influenza virus (strain A^1 Budapest 4/49) recoverable from inhibitor-virus mixtures after incubation for different periods by adsorption to and elution from chicken erythrocytes. Arithmetic means calculated from five experiments

A, 128 *HA* units of virus + 64 *HAI* units of α-inhibitor; *B*, 128 *HA* units of virus + 64 *HAI* units of α-inhibitor, coupled to *p*-phenylenediamine; *C*, 512 *HA* units of virus + 64 *HAI* units of α-inhibitor, coupled to *p*-phenylenediamine; *D*, 128 *HA* units of virus + 3 mgm./ml. *p*-phenylenediamine

HA unit = the amount in 1 ml. of virus suspension causing partial hæmagglutination; *HAI* unit = the amount in 1 ml. which partially inhibited hæmagglutination produced by 1 ml. of Lee virus containing 4 *HA* units

Fig. 1,*B* illustrates an experiment with the *p*-phenylenediamine derivative of α-inhibitor. To prepare this substance, 3 mgm. of the lyophilized α-inhibitor was taken up in 1 ml. buffer solution and coupled to 3 mgm. *p*-phenylenediamine. (The lyophilized material could not be redissolved completely.) When virus was added to this modified inhibitor, a sudden decline in the virus recoverable from erythrocytes occurred. If high-titre viral preparations were used, some rise was observed later (Fig. 1,*C*), but when the virus preparation had an initial titre comparable with those used in the above experiments, no virus could be demonstrated in the eluates during the 6-hr. period of experiment (Fig. 1, *B*). The total disappearance of virus, which has been demonstrated in numerous experiments, could not be attributed to either the α-inhibitor or the uncoupled chemical compound alone (Fig. 1, *A* and *D*).

The inhibition of virus multiplication was examined by Horváth's roller-drum micro-technique[10]. To determine the strength of the inhibitor several dilutions were tested. First, the inhibitor dissolved in 0·5 ml. medium was placed in each vial, then the allantoic membrane fragments in 0·1 ml. were added, and finally 0·1 ml. of the *Bp* strain of virus. The infective titre of the virus alone was also determined as a control. Eight cultures were inoculated with every dilution. After 72-hr. incubation, each culture medium was titrated for hæmagglutination. The inhibition of viral multiplication was expressed as the percentage difference between the arithmetic mean of hæmagglutination titres in the control vials and that for the experimental group. The inhibitory potency of a substance was characterized by the dose which caused a 75 per cent inhibition (D_{75}). In Table 1, D_{75} is expressed in hæmagglutination inhibition units. (One such unit is present in 1 ml. of a preparation which partially inhibits the hæmagglutination of a suspension containing 4 hæmagglutination units Lee virus, in the indicator state, per ml.) In order to estimate the reproducibility of the index D_{75}, that for the diethyl-*p*-phenylenediamine-diazonium-α-inhibitor was determined against 10^4 ID_{50} in five experiments. The coefficient of variation as multiplied by Student's value was found to be ± 55 per cent. In the vials containing too little inhibitor to cause any inhibition the hæmagglutination titres slightly exceeded the control ones. This slight rise in titre was reproducible and found in every experiment. Regardless of this, the relation of inhibitor concentration to the percentage inhibition appeared to be approximately rectilinear and was scarcely influenced by the strength of the inoculum.

According to the D_{75} values determined against three strains (Table 1), preparations Nos. 3, 8 and 1 are considerably more potent (2·75–255-fold) than the control preparation. It was a general observation that different strains were differently inhibited by the same compound. Some preparations, such as No. 14, were even ineffective with one or two strains while definitely effective with others. The inhibition of multiplication by larger doses of the control preparation might be explained by thermo-inactivation occurring while the virus is fixed to the inhibitor.

To obtain some information on the mechanism of inhibition, preparation No. 8 was added to an excess of active virus, kept at room temperature for 48 hr., centrifuged at 20,000 *g* for 1 hr., and the supernatant was heated to 90° C. to inactivate any virus possibly present. The heated supernatant failed to inhibit virus, indicating that the inhibitor had sedimented in combination with virus. (Free inhibitor was not sedimented in control experiments; heating to 90° failed to inactivate inhibitor.)

The inhibitory effect of substance No. 8 has been studied in the embryonated egg. Sixty eggs were infected with 10^5 ID_{50} of the *Bp* strain. Twenty eggs were each given 0·5 ml. of a solution containing 48 hæmagglutination inhibitor units 30 min. before infection, another twenty the same dose 30 min. after infection. The remainder served as control. After 48 hr. incubation the infective titres in the three pools of allantoic fluids were $10^{-7·4}$, $10^{-7·5}$ and $10^{-9·2}$, respectively. A similar inhibition (2·0 $\log_{10}$ units) was demonstrated with the Lee strain as test virus.

Thus it is concluded that some modified preparations of α-inhibitor, being as avid for the influenza virus as α-inhibitor itself, combine with the virus more firmly than α-inhibitor does. Marked reduction in the amount of virus multiplication by preparation No. 8 has been demonstrated in the egg with two strains of virus even if inhibitor was injected 30 min. after infection. The modified inhibitors are expected to help us in studying the role of cell receptor in the multiplication of myxoviruses. Similar derivatives of neuramic acid esters might be of more practical importance.

This work will be published in detail in the *Acta Microbiol. Hung.* (Budapest).

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ACTION OF *VICIA FABA* ON ERYTHROCYTES: POSSIBLE RELATIONSHIP TO FAVISM

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THE disease favism is a hæmolytic anæmia which results from the ingestion of the broad bean (*Vicia faba*) by some people. The disease has been recognized in most countries around the Mediterranean Sea for many years, and its clinical manifestations have been well documented[1]. Work both in Italy[2] and Israel[3] has shown that the disease only occurs in persons who have an inherited abnormality of the erythrocytes in that there is a deficiency of the enzyme glucose-6-phosphate dehydrogenase. This basic deficiency is reflected in a lowered level of the amount of reduced glutathione in the red blood cells and an instability of the glutathione in the presence of certain substances such as acetylphenylhydrazine, aniline, hydroxylamine, α- and β-naphthol and the anti-malarial drug primaquine[4]. Such agents cause both *in vitro* and *in vivo* breakdown of erythrocytic glutathione, whereas agents such as ascorbic acid and cysteine cause *in vitro* destruction only[5,6].

We recently reported[7] that extracts prepared from fresh beans of *Vicia faba* show a selective effect upon the glutathione of erythrocytes from sensitive subjects, that is, those possessing the genetic abnormality. This work has now been extended to show that the active agent of *Vicia faba* is present also in extracts of pollen and pistils, that the effect on glutathione can be demonstrated simply by incubating whole blood of sensitive subjects with whole beans and that the effect is not due to common reducing agents such as ascorbic acid and cysteine.

Table 1 shows the effect of incubating 1-ml. blood samples from both sensitive and non-sensitive subjects with 0·1 or 0·2 ml. amounts of extracts of pollen and pistils. Blood glutathione-levels were measured before and after incubation[6]. The pollen extracts were prepared by suspending 1 vol. of loose pollen in 10 vol. of saline at 4° C. for 1 hr. followed by centrifugation at 500 r.p.m. for 2 min. Pistil extracts were prepared in a similar manner using 5 vol. of saline. Fresh young beans (approx. 8 mm. in maximum diameter) were used in the whole-bean experiments. In all three cases, the incubations showed a significant *in vitro* effect on blood from sensitive subjects compared with non-sensitive subjects.

Table 1. EFFECT OF EXTRACTS OF POLLEN AND PISTILS AND OF WHOLE BEANS ON ERYTHROCYTIC GLUTATHIONE

Source of activity	Vol. of extract used (ml.)	Subjects	No. of samples	Glutathione (mgm. per 100 ml. red blood cells) before incubation	after incubation	*P* value on basis of '*t*' test
				Mean S.D.	Mean S.D.	
Pollen extract	0·2	Sensitive	10	33·2 ± 5·0	21·3 ± 3·2	0·01
		Non-sensitive	8	55·3 ± 8·3	48·0 ± 6·3	
Pistil extract	0·1	Sensitive	10	36·1 ± 4·8	26·8 ± 4·2	0·001
		Non-sensitive	10	56·5 ± 6·0	52·6 ± 7·0	
Whole bean	—	Sensitive	10	36·2 ± 5·3	28·4 ± 5·0	
		Non-sensitive	10	61·8 ± 8·8	61·9 ± 9·1	

This effect cannot be due to cysteine because this would give a colour with the nitroprusside reaction used for estimating glutathione. Control experiments showed that the addition of extracts to blood followed by immediate precipitation of the proteins and glutathione estimation (with no incubation period) has virtually no effect upon the apparent blood glutathione-level. Hence the extracts do not contain significant amounts of cysteine. Further, the effect of the extracts is not due to the action of ascorbic acid. To demonstrate this, an extract of fresh young beans was prepared using ½ vol. of saline by the method previously described[7]. The action of 0·2 ml. of this extract (which contains not more than 3 μgm. of ascorbic acid in the 0·2 ml., an amount far below the concentrations reported to have an *in vitro* effect on erythrocytic glutathione[5]) was compared with the effect of incubating 1 ml. of blood with 2 mgm. of ascorbic acid in 0·2 ml. of saline. With an incubation time of 10 min. only the *Vicia faba* extract had a marked effect upon erythrocytic glutathione (Table 2).

These experiments show, therefore, that *Vicia faba* pollen and pistils, in addition to the bean, itself contain an active principle which has a marked effect on the erythrocytic glutathione of sensitive subjects. The action is not due to ascorbic acid or cysteine so that a specific effect of *Vicia faba* is still indicated. This direct action on the red blood cells *in vitro* suggests a way in which ingestion of the bean or inhalation of the pollen might result in *in vivo* hæmolysis.

It remains to demonstrate that the effects shown in this article are specific for *Vicia faba* and are not given by other vegetables at least to the same extent. Whole fresh peas, incubated as in the whole-bean experiments, showed no effect. Certain other vegetables tried, for example, runner beans, caused massive agglutination of the erythrocytes. When this occurs, false low glutathione-levels are obtained, probably due to lack of complete hæmolysis which is necessary for accurate estimation of the whole

Table 2. EFFECTS OF *Vicia faba* EXTRACT AND OF ASCORBIC ACID ON GLUTATHIONE OF THREE DIFFERENT SAMPLES OF 'SENSITIVE' ERYTHROCYTES

Before incubation	After incubation with ascorbic acid	After incubation with *Vicia faba* extract
42*	43	29
34	35	24
44	44	28

* All values are in mgm. glutathione per 100 ml. red blood cells. The incubation time was 10 min.; for details see text.

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blood glutathione-level. With *Vicia faba* extracts, we have noted non-specific falls in glutathione if added to non-sensitive blood containing insufficient ACD. In these instances coarse clumps of blood were observed. This effect is abolished with the addition of further anticoagulant. Only those results obtained in the absence of agglutination are therefore valid for the purposes of comparison. In our experience, *Vicia faba* extracts are the only ones so far tested which give a statistically verified selective *in vitro* action on erythrocytic glutathione of sensitive subjects.

Though these results in no way prove that favism is caused by a direct action of an agent in *Vicia faba* on erythrocytes by initiating hæmolysis, they strongly suggest that such a possibility should be tested *in vivo*. Such a direct action appears to offer an attractive alternative hypothesis as to how sensitive subjects may develop a hæmolytic anæmia following ingestion of the broad bean, although the evidence for allergy in addition to the genetic erythrocytic defect cannot be excluded[8].

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A GENE DETERMINING PRESENCE OR ABSENCE OF ε-N-METHYL-LYSINE IN *SALMONELLA* FLAGELLAR PROTEIN

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ACCORDING to current views on the gene control of protein structure[1], the amino-acid which is to constitute a particular link in the polypeptide chain of a given protein is specified by a group of bases in a corresponding region of the deoxyribonucleic acid (DNA) molecule of a gene which determines the structure of this protein. Only twenty amino-acids, all in the L configuration if optically active, are common constituents of proteins, and it has been suggested that only twenty different base-groups occur in DNA which specify amino-acids. This hypothesis predicates: (*a*) that the presence of an uncommon amino-acid in a protein always results from some cause other than its specification by a group of bases in the DNA of the gene determining the amino-acid sequence of the protein (for example, from conversion of a common amino-acid into an uncommon one after its incorporation in a polypeptide chain); and (*b*) that heritable variation in respect of the presence or absence of an uncommon amino-acid in a particular protein must result from the action of a genetic determinant other than the gene determining the amino-acid sequence of this protein. An experimental test of predicate (*b*) has now been made in respect of the uncommon amino-acid ε-N-methyl-lysine, which Ambler and Rees[2] reported as a constituent of the flagellar protein ('flagellin') of some bacteria of the genus *Salmonella*; in the material they examined lysine and N-methyl-lysine were about equally abundant. We have now found that N-methyl-lysine is absent from some *Salmonella* flagellins; and by genetic analysis, using phage-mediated transduction[3], we have located a gene determining the presence or absence of N-methyl-lysine in flagellar protein.

Flagellins from many *Salmonella* strains of various species (serotypes) were tested for N-methyl-lysine by paper electrophoresis of acid-hydrolysed flagella with results to be reported in detail. In the case of diphasic strains (that is, strains able to produce flagella of either of two distinct antigenic types) flagella were collected from cultures in phase 1 and from cultures in phase 2. In each of the ten diphasic strains tested, the two flagellins obtained were alike in respect of presence or absence of N-methyl-lysine. Seven strains of *S. typhimurium* (phase 1 antigen *i*; phase 2 antigen 1,2,3) were all positive for N-methyl-lysine; four strains of *S. paratyphi B* (antigens *b* and 1,2) were all negative for N-methyl-lysine. But of thirteen strains of *S. derby* (phase 1 antigen *f,g*; no second phase), four were negative for N-methyl-lysine, the rest being positive.

We next tested flagellins from a series of motile derivatives of *SW* 543, a non-flagellated monophasic strain of *S. paratyphi B* with the (latent) phase 1 flagellar antigen *b*. The flagellar protein of a flagellated mutant of this strain lacked N-methyl-lysine, like the flagellins of other strains of *S. paratyphi B* tested. Strain *SW* 543 lacks flagella because of mutation at a *fla* locus close to the locus which regulates the phase 1 flagellar antigen, and motile clones obtained from it by transduction of the *fla*+ gene from a motile donor strain, are of two sorts: one sort show flagellar antigen *b*, and are attributed to replacement of only the *fla*− gene of strain *SW* 543 by its wild-type allele from the donor strain; the other sort have the phase 1 flagellar antigen of the donor strain, and are attributed to replacement of both the *fla*− gene and the linked gene determining the phase 1 flagellar antigen *b* of strain *SW* 543 by their alleles from the donor strain[4]. Of nine motile transductant clones of the latter type, with phase 1 flagellar antigens derived from various donor strains, eight were positive for N-methyl-lysine; the donor

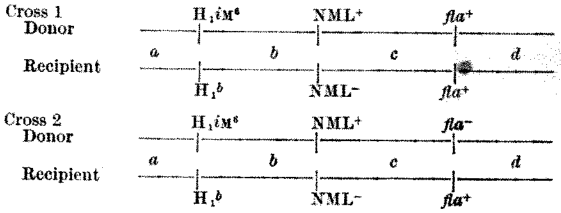
Table 1. ANTIGENIC CONSTITUTION AND N-METHYL-LYSINE CHARACTER OF THE FLAGELLA OF RECOMBINANTS OBTAINED BY TRANSDUCTION OF FLAGELLAR ANTIGEN-DETERMINANTS BETWEEN DIPHASIC FLAGELLATED STRAINS

Strain Description	Clone No.	Phase 1 flagella		Phase 2 flagella	
		Antigen	NML	Antigen	NML
S. typhimurium wild-type parent strain	*LT* 2	*i*	+	*1,2,3*	+
S. abony wild-type parent strain	*SW* 803	*b*	–	*e,n,x*	–
S. typhimurium given $H_1{}^b$ from *S. abony*	*SW* 699	*b*	–	*1,2,3*	–
S. typhimurium given $H_1{}^b$ from *S. abony*	*SD* 7	*b*	–	*1,2,3*	–
S. typhimurium given $H_2{}^{e,n,x}$, from *S. abony*	*SW* 698	*i*	+	*e,n,x*	+
S. abony given $H_1{}^i$ from *S. typhimurium*	*SW* 933	*i*	+	*e,n,x*	+
S. abony given $H_2{}^{1,2,3}$, from *S. typhimurium*	*SW* 932	*b*	–	*1,2,3*	–

strain concerned in the case of the exceptional, N-methyl-lysine - negative recombinant was found to be itself negative for N-methyl-lysine. By contrast, ten clones of the first type, that is, with antigen *b*, each obtained by transduction of the *fla*$^+$ gene from a N-methyl-lysine - positive strain, were all negative for N-methyl-lysine. These results indicate that the presence or absence of N-methyl-lysine in the flagellar protein of a strain in phase 1 is controlled either by the gene which determines the antigenic character of this protein or by a closely linked gene.

The phase 1 and phase 2 flagellar antigens of diphasic strains are controlled, respectively, by two series of alleles, at loci termed H_1 and H_2, which in phage-mediated transduction appear unlinked[5]. A series of hybrids obtained by transduction of one or other of these genes between flagellated diphasic strains differing both in N-methyl-lysine character and in their phase 1 and phase 2 flagellar antigens were next examined. Table 1 records the results obtained on testing recombinants of a strain of *S. typhimurium* (positive for N-methyl-lysine; flagellar antigens *i* and 1,2,3) and a strain of *S. abony* (negative for N-methyl-lysine; antigens *b* and *e,n,x*); less extensive experiments on other strains gave similar results. It was found: (i) that the antigenically distinct phase 1 and phase 2 flagellins produced by a diphasic recombinant strain were always alike in respect of the presence or absence of N-methyl-lysine; and (ii) that when the phase 1 flagellar antigen of a strain was altered by transduction, the N-methyl-lysine character of the recombinant was always the same as that of the donor strain from which the phase-1 antigenic determinant (H_1) had been transduced; whereas replacement of the phase 2 determinant (H_2) of a strain by transduction from a strain of opposite character had no effect on the N-methyl-lysine character of the recipient strain.

These results indicated that the presence or absence of N-methyl-lysine in the flagellin(s) of a *Salmonella* strain is decided by a genetic determinant which is either closely linked to, or part of, the H_1 locus. To decide between these alternatives, a search was made for re-assortment of phase 1 antigenic character and N-methyl-lysine character during transduction. The recipient strain was a monophasic flagellate strain of *S. paratyphi B* (phase 1 antigen *b*, no second phase) and negative for N-methyl-lysine. Two donor strains were used: in cross 1, a sub-line of *S. typhimurium* strain *LT*2, positive for N-methyl-lysine, diphasic, the phase 1 antigen being *i M*6 (a mutant derivative of *i*: Joys, T. M., unpublished work); in cross 2, a non-motile mutant of the *i M*6 strain, lacking flagella because of mutation at a site closely linked to H_1. Motile transductants with the phase 1 flagellar antigen of the donor strain were selected by inoculating the phage-treated recipient bacteria into semi-solid medium containing sufficient anti-*b* serum to prevent the spread of motile bacteria with antigen *b*. From cross 1 we tested sixteen clones with antigen *i M*6, resulting from transduction of the H_1 locus from the motile donor strain; all were positive for N-methyl-lysine, like the donor strain. From cross 2 we tested ten clones with antigen *i M*6, resulting from incorporation by recipient bacteria of the H_1 gene from the non-motile donor but not the closely linked *fla*$^-$ gene causing its non-motility; eight were positive for N-methyl-lysine, like the donor, while two were negative, like the recipient. This indicates the existence of a locus for N-methyl-lysine character which is closely linked to H_1, but separable from it, probably lying between H_1 and the gene causing absence of flagella in the non-motile donor strain used:



In cross 1 motile transductants with antigen *i M*6 can be produced by incorporation of a single transduced chromosome fragment by one cross-over in region *a* and one in region *b*, *c* or *d*; if *b* is much shorter than *c* plus *d*, then nearly all such transductants will have the N-methyl-lysine character of the donor strain. In cross 2 one cross-over in *a* and one in *b* or *c* are required; if *b* is not very much shorter than *c*, a substantial proportion of the motile recombinants with antigen *i M*6 will have the N-methyl-lysine character of the recipient strain.

Thus the presence or absence of N-methyl-lysine in the flagellar protein produced by bacteria in flagellar antigenic phase 2 is determined by a gene which is not closely linked to the H_2 gene determining the antigenic character of this protein; and in the case of bacteria in phase 1 by a gene closely linked to, but separable from, the H_1 gene determining the antigenic character of the protein produced. The available evidence suggests that *Salmonella* flagella are composed of a single protein. The H_1 and H_2 genes presumably specify not only the antigenic specificities of the phase 1 and phase 2 flagellar proteins but also their complete amino-acid sequences, because flagella of different antigenic types differ in some physical properties, in relative abundance of certain amino-acids and in the peptide 'maps' given by their enzymatic digests (Ambler and McDonough, unpublished results). If this is so, then the control of the presence or absence of N-methyl-lysine in these proteins by a gene other than H_1 or H_2 is in accordance with predicate (*b*) above, that the genetically determined presence or absence of an uncommon amino-acid in a particular protein cannot be controlled by the gene which specifies the amino-acid sequence of this protein. The experimental results are thus compatible with the hypothesis that only twenty different base-groups specifying amino-acids occur in DNA.

The proteins of *Salmonella* other than flagellar protein do not contain N-methyl-lysine in detectable amounts. The gene determining the presence of N-methyl-lysine in flagellin, but no other protein, perhaps causes the production of an enzyme which methylates the ε-amino-groups of certain lysine radicals in flagellin already formed. Experiments to test this hypothesis are being made.

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CYTOLOGY OF CELLS WHICH SYNTHESIZE PROTEIN

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THE cells found in glands which synthesize and secrete protein have been very extensively studied by biochemists and cytologists with the object of discovering the cellular processes associated with protein synthesis. In this article we wish to describe and comment on the fine cytology of another, but less-studied, class of cells which synthesize protein. These, in contrast to secretory cells, retain their product within their plasma membrane and do not secrete it. Examples are the cells of the mammalian epidermis which make keratin, the myoblast which forms the muscle proteins and the erythroblast which forms hæmoglobin. For want of a better name we shall call these cells 'retaining cells'. As well as these examples of cells which synthesize specific proteins there are others which have a similar cytoplasmic structure. These are cells with a high rate of growth, for example, animal embryonic cells, plant meristematic tissue, bacteria and many tumours. In these cases the protein synthesized is their own cellular protein.

The Secretory Cell

Examination on the electron microscope of thin sections of secretory cells has revealed in many of them systems of membranes to which are attached numerous dense particles 100-200 Å. in diameter (Fig. 1*a*). The almost simultaneous discovery of these structures by several groups, who each introduced their own terms, has produced a complex nomenclature. The two most commonly used terms, 'endoplasmic reticulum'[1] and 'ergastoplasm'[2], are a source of further confusion, in that, whereas some authors use them to describe membranes with particles, others include those membranes in the cell which are 'smooth' or 'agranular' and are not covered by particles. The presence in the cell of these different types of membranes make us, at least for the present, favour the Sjöstrand nomenclature[3], which defines the particle-covered membranes as 'α-cytomembranes', the external plasma membrane and infoldings of it as 'β-cytomembranes', and the smooth membranes found in the Golgi zone of the cell as 'γ-cytomembranes'. The particles themselves have at least three names (namely, Palade, RNP or microsomal particles). The use of the terms 'microsomes' and 'ribosomes' is inadvisable since these terms are operational terms for specifying biochemical fractions and are not therefore synonymous with morphological structures.

Porter[4] was one of the first to point out the correlation between α-cytomembranes in a cell and basophilia. The integrated biochemical and electron microscopic studies of Palade and Siekevitz[5] showed that α-cytomembranes are the main component of the microsome fraction of the cell, and furthermore that the particles are found in the ribosome fraction. It was therefore inferred that the particles are the site of much of the cytoplasmic ribonucleic acid, the relationship of which to protein synthesis is generally accepted[6,7].

The presence of the particles in these cells is therefore understandable, but the function of the membranes is more obscure. Some have suggested[8,9] that they may provide an interconnected pathway in the cell which is required to guide both raw materials and products of protein synthesis from one site in the cell to another. Inter-connexions between the different membrane systems are occasionally seen; however, evidence that all the membranes are connected (that is, to form a reticulum), which

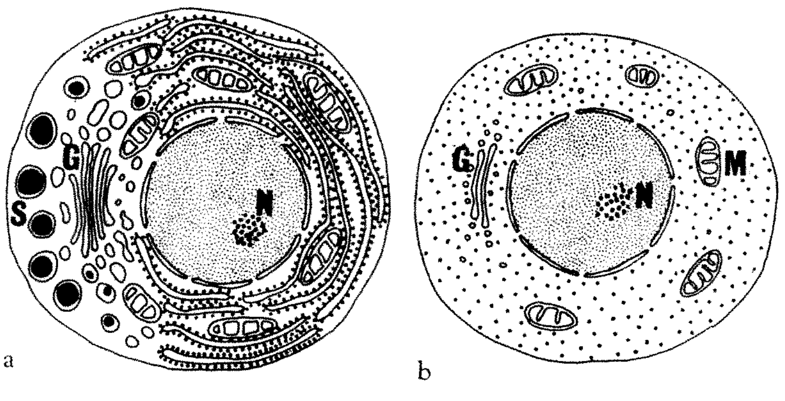

Fig. 1. The fine cytology of the two kinds of cells forming protein: (*a*) the secretory cell, and (*b*) the retaining cell. *M* are mitochondria, *N* nucleoli within the nuclei, *G* clusters of Golgi membranes. The characteristic organelle of the secretory cell is the α-cytomembrane or membrane covered with particles. In the secretory cell the particles are free and there are few α-cytomembranes. In the latter type of cell the synthesized protein appears freely throughout the cytoplasm and is not contained in vesicles

could only be obtained from serial sections, is still lacking. The membranes could also function as a support to localize enzymes[10]; they would represent the cytoskeleton described by Peters[11]. A recent comprehensive review of these structures by Haguenau[12] covers both the microscopical and the biochemical observations.

Nearly all the electron micrographs of these structures have been made after using osmium tetroxide as a fixative, which is particularly satisfactory for delineating both membranes and particles. Other fixatives give different images; for example, permanganate fixation[13] reveals only the membranes, and formalin fixation (unpublished work) shows only the particles; in this latter case the particles are arranged in sheets, so that one would infer that the membranes are preserved but lack sufficient electron density to be visualized. Recent observations by Sjöstrand and Hanzon[14] on frozen-dried tissue have indicated that, after this method of fixation, no particles are seen, but instead a continuous layer of electron-dense material. Assuming that this method of fixation is the most likely to preserve the structure of the living cell, it has been suggested that the particles are an artefact of osmium fixation. However, the similar and regular size of the particles as shown both by electron microscopy and by ultracentrifugation makes it unlikely that they are a random aggregate of material. An alternative explanation would be that the particles extend over a much larger volume in the cell than the micrograph of the specimen fixed with osmium tetroxide would suggest; this would be expected if they had a large degree of hydration.

Retaining Cells

The successful correlation of many structural aspects of secretory cells with biochemical data has tended to obscure the fact that it applies only to this particular class of cells. The other group of protein-synthesizing cells, the retaining cells, prove to have a very different appearance in the electron microscope, for in these the elaborate system of membranes covered with particles (α-cytomembranes) is not present (Fig. 1*b*). Dense particles similar to those found on the α-cytomembranes in secretory cells are numerous, but in retaining cells they are freely dispersed in the cytoplasm. The distribution of particles corresponds to the diffuse basophilia of these cells.

We originally observed that the cells of the hair cortex, where the fibrous protein keratin is synthesized and retained in the cell, had this type of cytoplasmic structure[15]. This led us to study other cell systems which also retain the protein they synthesize. The cells studied are noted in Table 1. From our observations we are able to make some general comments about these cells.

Retaining cells are not devoid of membranes; a nuclear membrane with typical 'pores', and a plasma membrane, are always present. Moreover, a cluster of smooth γ-membranes in the Golgi region of the cell is usually seen. Indeed there is much evidence for regarding this system of membranes as a universal cell structure. However, the characteristic feature of these cells is always the large number of free particles; from this it follows that these particles, which are not necessarily attached to membranes, are the essential cytoplasmic element associated with protein synthesis. Another feature of these cells is that the protein, the product of synthesis, is not enclosed in a membrane; whereas in secretory cells a membrane either encloses the secretory granule or alternatively the protein appears inside the distended vesicles of the α-cytomembranes (*S*, Fig. 1*a*). The absence of limiting membrane in retaining cells is perhaps connected with the fact that the protein does not pass through the plasma membrane.

Table 1

	Type of cell	Product
Cells synthesizing specific protein	Epidermal cells:	
	skin	Keratin
	hair cortex	Keratin
	hair inner root sheath	Keratohyalin
	Erythroblasts	Hæmoglobin
	Developing muscle cells	Actomyosin
Rapidly proliferating cells	Embryonic cells	General cellular protein
	Tumour cells	
	Lymphocytes	
	Meristematic plant cells	
	Bacteria	

The examples of retaining cells in Table 1 may themselves be divided into two classes. The first examples are cells the cytoplasm of which becomes filled with a specific protein. During the period of protein synthesis the cells contain free particles, but eventually the cytoplasm becomes completely filled with the protein and the particles disappear. The resultant cell is a fully differentiated one, and does not afterwards divide. The second group of cells are those with a high rate of cell division; the protein synthesized in these cells is the cellular protein required for growth. This second type of retaining cells includes several groups of cells, which it is convenient to discuss separately.

Embryonic and Tumour Cells

Embryonic cells and ova have a mixed cytoplasmic pattern (Mercer, E. H., and Wolpert, L., unpublished work), both free particles and particles attached to membranes being present. Free particles at first predominate, but as cleavage and differentiation proceed, a separation into secreting and retaining cells emerges. (See also Slauterback and Fawcett[15a].)

Tumour cells may be of either type. Tumours derived from retaining type cells, for example, epidermal carcinomas (Mercer, E. H., unpublished work), have the corresponding cytoplasmic structure. Primary tumours, which are derived from secretory cells and which are sufficiently well differentiated to retain their secretory ability, for example plasma cell tumours[16], contain α-cytomembranes. Those tumours, however, although perhaps originally well differentiated, that have lost their secretory ability, are of the retainer type[17]. These would include most of the anaplastic animal tumours maintained by serial grafting, both in the solid and in the ascites form, as our own work has shown (Mercer, E. H., and Easty, G. C., unpublished work). This reversion of tumour cells to the cytoplasmic pattern of embryonic cells correlates with many of their other properties.

Plant Tissues

Plant meristem tissue contains high concentrations of free particles[18] and may thus be regarded as of the retaining-cell type. Some reports[19,20] claim the presence of endoplasmic reticulum, but the use of this all-inclusive term fails to make clear whether the authors refer to α-membranes covered with particles or γ-membranes with smooth surfaces. Our own observations (Mercer, E. H., and Mercer, F. V., unpublished work) on developing root tips (maize) reveal small amounts of α- and γ-cytomembranes in some cells, suggesting that initially the cell cytology is generalized. Because plant cells do not commonly secrete protein, the absence of α-cytomembranes is to be expected; their occurrence in those rare plant cells which do secrete would be added corroboration of their required presence in secretory cells. We have examined (with Prof. W. O. James and Dr. R. M. Leach, Botany Department, Imperial College) the scutellum of germinating barley seeds. These cells produce the enzymes required for the breakdown of the endosperm. Our results show that the cytoplasm of these cells has an appearance nearer that of animal secretory cells than that of typical plant cells. Membranes covered with particles are present, although some free particles exist also.

Bacteria

Recent electron micrographs of bacteria[21,22] show free particles embedded in the dense amorphous cytoplasmic region of the cell. It is possible that the particles are smaller than those in animal cells, but the good correlation between these particles and the isolated ribosomes[23] is sufficient to regard them as a site of RNA in the cell and therefore analogous to the particles in animal cells. Bacteria therefore have the cytoplasmic pattern of retaining cells, although their cytology in other respects is very different: the absence of a nuclear membrane and of mitochondria is most obvious. Brieger *et al.*[24] have described membranes in some Mycobacteria, but these are of the smooth type. Although the presence of free particles agrees well with the rapid growth-rate of bacteria, the absence of membranes makes it difficult to explain bacterial secretion. That enzymes do appear in the extracellular medium is undoubted; either the mechanism of secretion must differ from that in animal cells or perhaps extracellular protein is liberated by lysis of the whole cell. This latter method of 'holocrine' secretion does occur in bacteria[25], but evidence that it is the only mechanism is lacking.

Protozoa

Many Protozoa contain elaborate organelles based on membranes, as might be expected from their complex intracellular differentiation. Two types of amœba studied by us are of interest. *A. proteus* has organelles based on smooth γ-membranes, but no α-cytomembranes[26]. The social amœbæ, *Dictyostelium* and *Polyspondylium*, which from their elaborate life-histories might be expected to secrete, have small amounts of α-cytomembranes[27].

Other Implications

The belief that all cells have an internal structure resembling that of the secretory cell is largely responsible for the speculation that all the membranes of the cell are interconnected to form a single system. The external plasma membrane may also be included in this system[28]. While this speculation is supported by evidence that all membranes have a common phospholipid-protein structure, and while transient interconnexions probably do occur, it is difficult to believe that there are any permanent interconnexions in retaining cells. The connexion between the cytoplasmic membranes and the nuclear membrane has been regarded as a means[29] by which nuclear RNP particles are transferred to the cytoplasm. The absence of this connexion in retaining cells, which nevertheless have a fenestrated nuclear membrane similar to that of secretory cells, makes it likely that some other mechanism of transfer of RNA must exist.

While nearly all the protein-producing cells yet examined belong to either of the two types we have described, we would not wish to imply that they represent the only two possibilities. In at least one tissue[30], the colleterial glands of the cockroach, part of which synthesizes an oxidase required to tan the structural protein of the egg case, there is a possibility that the enzyme is produced by mitochondrial proliferation. If this were so, it would be similar to the protein synthesis which occurs in mitochondria[31], which does not appear to contain RNP particles.

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LETTERS TO THE EDITORS

RADIOASTRONOMY

Secular Variation of the Flux Density of the Radio Source Cassiopeia A

In 1951, Ryle and Elsmore[1] reported on a series of observations of radio sources using an interferometer at a frequency of 81·5 Mc./s. A comparison of the flux densities of about one hundred sources measured nearly every day for eighteen months revealed no long-term variations greater than 10 per cent, or for some of the more intense sources, greater than 5 per cent.

It is now believed that the majority of sources are extra-galactic and would therefore not be expected to show any intrinsic variations over a few years only. A number of sources, however, have been identified with the remnants of galactic supernovæ of comparatively recent origin, and Minkowski[2] has shown that the intense source Cassiopeia A is probably of this type. Optical study of this source is difficult because of heavy obscuration in the region, but his measures of the velocities of the faint visible filaments suggest that the supernova exploded 258 ± 14 yr. ago. There is clearly the possibility of being able to detect a change in the intensity of this source.

Some observations for this purpose were carried out in Cambridge in 1956 with apparently positive results, but it was felt that further confirmation was required. This has recently been obtained and reveals that the flux density is decreasing by about 1 per cent per annum. Similar measurements are also in progress at the U.S. National Radio Astronomy Observatory, Green Bank (J. Findlay, private communication), at a frequency of 1,420 Mc./s.

Absolute measurements of flux density are not easy, but fortunately the flux density of Cassiopeia A (at $\alpha = 23$h. 21m., $\delta = +58° 32'$) may readily be compared with that of Cygnus A (at $\alpha = 19$h. 58m., $\delta = +40° 36'$), since these sources are the strongest two at metre wave-lengths and are not far distant from one another in the sky. Cygnus A has been identified with a pair of colliding galaxies and its flux density is not likely to change appreciably in a time small compared with that taken by radio waves to traverse the source itself—say 10^5 years.

The same aerial[3] has been used for all the observations, which have been at a frequency of 81·5 Mc./s. It is a fixed transit instrument consisting of two broadside arrays, each measuring 10 × 1 wave-lengths, separated by 100 wave-lengths on an east–west axis. The reception pattern of each aerial is therefore restricted to ± 3° in an east–west plane, but covers about ± 45° in the north–south plane. There are three sets of observations, one in December 1948 and January 1949, one in November 1956 and one in December 1960. The receiver used for the first two series was a total-power receiver of the type developed by Machin, Ryle and Vonberg[4] and for the third series, a phase-switching receiver[5]. In each case, records showing obvious signs of scintillations have been discarded, leaving records of 14, 10 and 13 days, respectively.

Table 1

Date	Mean ratio of observed record amplitudes Cassiopeia A/Cygnus A	Standard error of mean
December 1948 January 1949	1·751	± 0·024
November 1956	1·635	± 0·019
December 1960	1·536	± 0·017

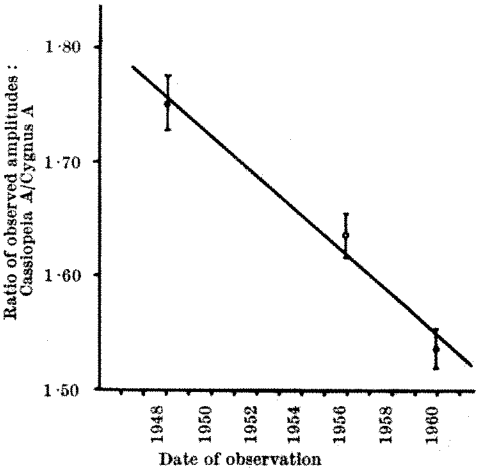

Fig. 1. A plot of the mean ratios of observed amplitudes of records of the radio sources Cassiopeia A and Cygnus A as a function of time. The vertical lines indicate standard errors

Table 1 gives the averages of the ratios of observed record amplitudes of Cassiopeia A and Cygnus A for the three sets of observations and in Fig. 1 they are plotted against the dates of the observations. The standard errors of the averages of the ratios are indicated on the plot. It should be noted that the ratios have *not* been corrected for the aerial polar diagram.

Unless some unknown systematic error varying with time is involved, there is very strong evidence for a change in the relative flux densities of Cassiopeia A and Cygnus A. If it is assumed that the flux density of the latter source has remained constant, then a least-squares solution for the best straight line through the points indicates a decrease in the flux density of Cassiopeia A of (1·06 ± 0·14) per cent per annum.

A number of possible sources of error are eliminated by the use of the same aerial system for all the observations. Some others that have been considered include non-linearity of the receivers, effects of a variation of output time constant (since the periodicities of the interference fringes of Cygnus A and Cassiopeia A differ) and changes in the polar diagram of the arrays. None is thought to be sufficient to account for the observed change of ratio. The effect of the increase of the angular size of the Cassiopeia A source with time is quite negligible.

Shklovsky[6], assuming that the radio radiation is due to the synchrotron mechanism, has made a theoretical prediction that the present yearly fractional change of the flux density of Cassiopeia A is :

$$\frac{\Delta S_\nu}{S_\nu} = -\frac{\beta}{\text{age in years}}$$

where $\beta = 2(1 + 2\alpha)$ and α, the spectral index, is given by: $S_\nu \propto \nu^{-\alpha}$. If $\alpha = 0{\cdot}8$ and the age is 258 yr., then:

$$\frac{\Delta S_\nu}{S_\nu} \simeq -2 \text{ per cent}$$

which is remarkably close to the observed figure. Some of his assumptions are debatable and it would not seem difficult to modify them to obtain an even closer agreement. Shklovsky's argument involves a constant energy spectrum for fast electrons and a magnetic field inversely proportional to the square of the nebular radius. This implies that the fractional change will be independent of frequency, as seen above, since β for Cassiopeia A is independent of frequency[7]. It will be interesting to see if observations at other frequencies confirm this.

In conclusion, the obvious point must be made that Cassiopeia A should no longer be considered as a standard source for the measurement of flux densities.

We thank Prof. M. Ryle for the loan of the records taken during the period December 1948–January 1949.

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RADIOPHYSICS

A New Method for the Calculation of *N*(*h*) Profiles from Ionospheric *h*′(*f*) Curves

RECENTLY[1-4], a lamination method has been developed for the calculation of electron density-height profiles ($N(h)$ profiles) from ionospheric virtual height-frequency curves ($h'(f)$ curves) with an electronic digital computer.

This method includes the evaluation of integrals of the 'group refractive index' μ' of the radio waves in the ionosphere. Generally, μ' is related to the (phase) refractive index μ through the equation:

$$\mu = \mathrm{d}(f\mu)/\mathrm{d}f \qquad (1)$$

where μ' and μ are functions of the frequency f of the wave and the electron density $N(h)$ in the ionosphere, which is a function of the height h above ground.

According to the magneto-ionic theory, μ is given, under the influence of the Earth's magnetic field $\mathbf{H}_0$, by the Appleton–Hartree formula as[5]:

$$\mu^2 = 1 - \frac{X}{1 - \frac{1}{2}Y_T^2/(1-X) + [Y_T^4/4(1-X)^2 + Y_L^2]^{1/2}} \qquad (2)$$

for the ordinary wave, where

$X = f_0^2/f^2$, $Y = f_H/f$, $Y_T = Y \sin\theta$, $Y_L = Y \cos\theta$, and θ is the angle between the wave normal and $\mathbf{H}_0$, ($|\mathbf{H}_0| = H_0$). $f_H (= e\mu_0 H_0/2\pi m)$ and $f_0 (= [Ne^2/m\varepsilon_0]^{1/2}/2\pi)$ are the gyro-frequency of an electron and the plasma frequency in the ionosphere in M.K.S. units.

An ionospheric $h'(f)$ curve for the ordinary wave is given by:

$$h'(f) = \int_0^{h_r} \mu'(f, f_0(h))\mathrm{d}h \qquad (3)$$

where $f_0(h)$ is used instead of $N(h)$ in μ', and h_r denotes the level where the wave is reflected, $f_0(h_r) = f$.

Assuming that $f_0(h)$ is a monotonic function of h, equation (3) becomes:

$$h'(f) - h_0 = \int_0^f \mu'(f, f_0)(\mathrm{d}h(f_0)/\mathrm{d}f_0)\mathrm{d}f_0,\ 0 \leqslant f_0 \leqslant f \qquad (4)$$

h_0 is the level where $f_0(h_0) = 0$, or $N(h_0) = 0$.

Equation (4) is the starting-point of the lamination method[1].

Since formula (2) for μ is rather complicated and μ' is related to μ by equation (1), the evaluation of integrals of μ' seems, even by the use of an electronic computer, very elaborate[4].

To avoid this difficulty, I have devised an improved method for the calculation of $N(h)$ profiles.

Integrating both sides of equation (4) with respect to f, over the range of $0 \leqslant f \leqslant f_1$:

$$\int_0^{f_1}[h'(f) - h_0]\mathrm{d}f = \int_0^{f_1}\mathrm{d}f \int_0^f \mu'(f, f_0)(\mathrm{d}h(f_0)/\mathrm{d}f_0)\mathrm{d}f_0 \qquad (5)$$

and changing the order of integration in the right side of equation (5):

$$= \int_0^{f_1}\mathrm{d}f_0(\mathrm{d}h(f_0)/\mathrm{d}f_0)\int_{f_0}^{f_1}\mu'(f, f_0)\mathrm{d}f$$

According to equation (1), the integral in the above expression is:

$$\int_{f_0}^{f_1}\mu'(f, f_0)\mathrm{d}f = f\mu(f, f_0)\Big|_{f_0}^{f_1} = f_1\mu(f_1, f_0) - f_0\mu(f_0, f_0)$$

Since $\mu(f_0, f_0) = 0$ by equation (2), equation (5) reduces to:

$$\frac{1}{f_1}\int_0^{f_1}[h'(f) - h_0]\mathrm{d}f = \int_0^{f_1}(\mathrm{d}h(f_0)/\mathrm{d}f_0)\mu(f_1, f_0)df_0$$

Or, putting

$$\frac{1}{f_1}\int_0^{f_1}h'(f)\mathrm{d}f = g(f_1),\ \text{and}\ f_1 \to f,$$

we obtain:

$$g(f) - h_0 = \int_0^f (\mathrm{d}h(f_0)/\mathrm{d}f_0)\mu(f, f_0)\mathrm{d}f_0 \qquad (6)$$

where $g(f_1)$ is then the average of $h'(f)$ within $0 \leqslant f \leqslant f_1$.

Equation (6) is equivalent to equation (4) and can be used for the calculation of $N(h)$ profiles after the same manner as the above-mentioned lamination or matrix method[1]; but equation (6) contains the integral of μ instead of μ' in equation (4), so the programming for an electronic computer by equation (6) will be, we believe, much easier than that of equation (4).

It should be noted that this method can also be used for the $h'(f)$ curve of the extraordinary wave.

Furthermore, as f approaches critical frequency f_c, the virtual height $h'(f)$ tends to increase infinitely and $h'(f_c)$ is often indefinite; but, if:

$$g(f_c) = \lim_{f_1 \to f_c}(1/f_1)\int_0^{f_1}h'(f)\mathrm{d}f$$

converges, the height of maximum electron density $h(f_c)$ can be determined by equation (6).

Physically speaking, if the $N(h)$ profile has a definite maximum point, $g(f_c)$ will converge; and vice versa.

μ' has the property that $\mu'(f_0, f_0) \to \infty$, but $\mu(f_0, f_0) = 0$; and this may be related to the properties of $h'(f_c)$ and $g(f_c)$. For example, if an approximation of $h'(f) \doteqdot A/(f_c - f)^m$ is valid near the critical frequency f_c, where A and m are constants greater than 0, $g(f_c)$ converges if $1 > m \geqslant 0$, but $h'(f_c)$ tends to infinity.

Since $g(f)$ in equation (6) should be a monotonic function of f, $h'(f)$ should also be monotonic; therefore, this method cannot be applied for an $N(h)$ profile which has a 'valley' in the electron density.

The scaling error ε of the $h'(f)$ curve would be given by:

$$\varepsilon = h'(f)_{\text{scaled}} - h'(f)_{\text{true}} = \delta h' + (\mathrm{d}h'(f)/\mathrm{d}f)\delta f \qquad (7)$$

where $\delta h'$ and δf are the height and frequency errors which are proper to the quality of the $h'(f)$ record, and are considered to be random variables.

Simpson's numerical integration method[6] will be adequate for the calculation of $g(f)$ from the scaled values of $h'(f)$, and if a sufficiently large number of n-points (including the value of h_0) are scaled from an $h'(f)$ record between $f = 0$ and $f = f_1$ near f_c, the relative error of $g(f)$ will decrease proportionally to the inverse square root of n. On the other hand, the $h'(f)$ curve then can be represented, for example, through the Lagrangian interpolation formula[6], by a polynomial of degree $(n - 1)$, passing through these n-points, by:

$$h'(f) = \sum_{m=0}^{n-1} a_m f^m, \quad 0 \leqslant f \leqslant f_1 \qquad (8)$$

Then:

$$g(f) = \sum_{m=0}^{n-1} a_m f^m/(m + 1)$$

so the component of degree m in $g(f)$ is diminished by a factor of $1/(m + 1)$, which is of higher order than the factor $1/n^{1/2}$ of the relative scaling error of $g(f)$.

This defect, however, seems to be offset by the property of $g(f)$, which is rather insensitive to the possible large scaling error of the $h'(f)$ record near the critical frequency f_c owing to a large value of $\mathrm{d}h'(f)/\mathrm{d}f$ in equation (7); and the behaviour of $h'(f)$ near f_c is, probably, responsible for the terms of higher degrees in equation (8).

Further details of this work will be reported elsewhere.

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PHYSICS

Influence of an Electric Field upon the Heat Transfer from a Hot Wire to an Insulating Liquid

If a non-uniform electric field is applied to a medium of dielectric constant K_1, containing a region of lower dielectric constant K_2, the latter will experience a force directed towards the region of lower field strength. This force is proportional to the difference between the two dielectric constants and the gradient of the square of the electric field. Now, when a liquid is heated, and expands, there is a resultant fall in dielectric constant, so that if the heating occurs in a region of high field, the heated liquid should be forced into regions of lower field strength.

In a recent experiment, we have been able to show that this effect can be used for enhancing the rate of heat transfer between a heated wire and an insulating liquid in which the wire is immersed.

The apparatus consists of a coaxial cell, in which the hot wire, which is at ground potential, is surrounded by a cylindrical electrode, to which the high voltage is applied. Measurements of the current flowing through the wire and the voltage drop along it are used for determining both the temperature of the wire and the power input. Under equilibrium conditions (attained in a few seconds), the heat input to the wire becomes identical to the heat transferred from the wire to the liquid.

The results given in Fig. 1 were obtained using hexane as the working fluid; the central electrode was a 0·1-mm. diameter nickel wire, and the outer electrode was a 3-cm. diameter nickel cylinder, 10 cm. in length.

In Fig. 1, the upper curve corresponds to conditions of zero applied field, and shows the loss of heat from the horizontal wire due to natural convection;

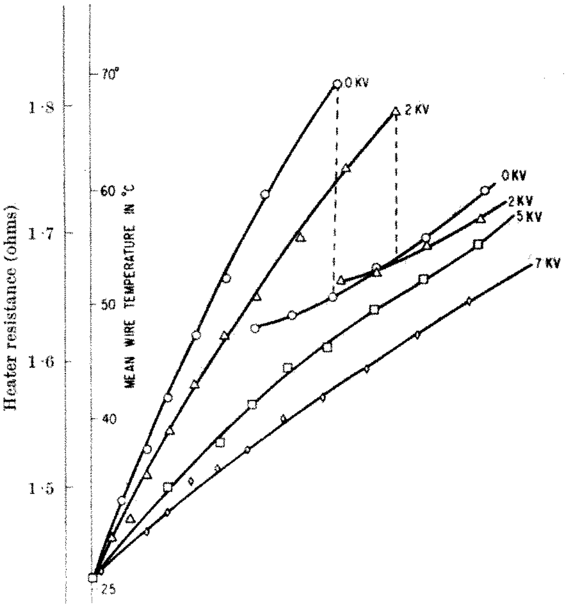

Fig. 1. Heat transfer from a 0·1-mm. diameter, horizontal nickel wire in n-hexane. Wire temperature versus power input for applied voltages of zero, 2, 5 and 7 kV., d.c. Ambient temperature 26° C.

the sudden break in the curve, at a power input of about 10 W., corresponds to the onset of boiling.

When a d.c. potential of a few kV. was applied between the wire and the cylinder, there was a fall in the temperature of the wire, as can be seen from the curves (Fig. 1), indicating a considerable enhancement of the heat transfer coefficient. (For example, below the region of boiling heat transfer, in going from zero to 5 kV., the mean temperature rise of the wire was approximately halved, indicating a doubling of the heat transfer coefficient.)

It is interesting to note that in the region of boiling heat transfer, bubbling of the liquid around the wire could be suppressed by application of the field; as can be seen from Fig. 1, this results in a decrease in heat transfer coefficient in going from zero to 2 kV.—though this effect is restricted to the immediate vicinity of the onset of boiling.

The results shown in Fig. 1 were obtained with a positive applied high voltage, but virtually identical results were found with a negative voltage. This observation is consistent with the initial assumption that the effect is an electrostatic one, and shows that the interactions between the electrostatic field and ions in the liquid play a negligible part in this phenomenon.

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Electron Microscopy of Crystal Lattices: an Anomalous Effect

AN explanation of intensity modulations in electron micrographs of crystal lattices[1] has recently been given by Labaw[2], and Hashimoto, Naiki and Mannami[3] have published a theoretical interpretation of fringe separation modifications which occur when a Bragg extinction contour lies in the same direction as the crystal 'lattice planes' resolved. The present communication deals with the conditions which exist when a Bragg extinction contour lies across the lattice planes in question. A theory given by Cowley and Moodie[4] is used to interpret a phenomenon which has recently been observed in practice (see Fig. 1).

Two strong beams only, the incident and one diffracted beam, are considered to have amplitudes U_0 and U_h respectively in the crystal. Then:

$$U_0 = [\cos\omega H - i(\pi\zeta_h/\omega)\sin\omega H]\exp\{i\sigma HE_0 - \rho HG_0 + \pi i\zeta_h H\} \qquad (1)$$

$$U_h = iF_h(\sin\omega H/\omega)\exp\{i\sigma HE_0 - \rho HG_0 - \pi i\zeta_h H\} \qquad (2)$$

where F_h is a structure factor:

$$F_h = \sigma E_h + i\rho G_h$$

and:

$$\omega = (\pi^2\zeta_h^2 + F_h F_{\bar{h}})^{1/2} \qquad \text{(see ref. 4b)}$$

In these equations, H is the crystal thickness and ζ_h is a factor representing the deviation from the Bragg angle, being the separation between the sphere of reflexion and the reciprocal lattice point given by h. In the Bethe[5] terminology, ζ_h is called the excitation error.

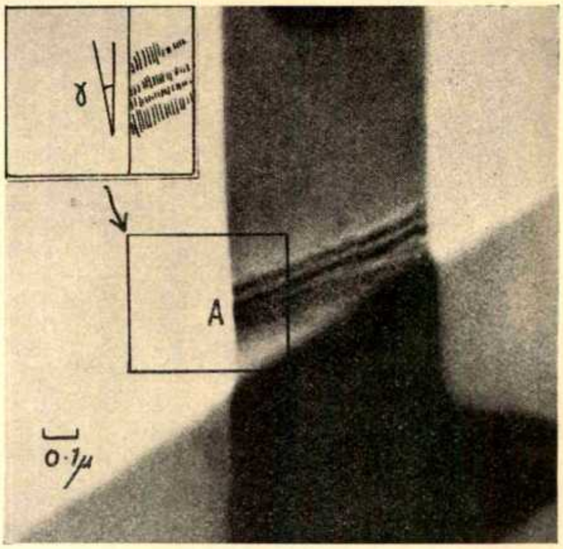

Fig. 1. Electron micrograph of a crystal of platinum phthalocyanine overlying a second crystal. $20\bar{1}$ fringes are resolved at *A* on the Bragg extinction contours. The inset is a diagrammatic representation of the area marked

Equations (1) and (2) enable one to obtain the intensity distribution at the exit face of the crystal:

$$\psi\psi^* = |U_0 + U_h\exp\{-2\pi ihx/a\}|^2$$

that is,

$$\psi\psi^* = 1 - 2|F_h|\frac{\sin\omega H}{\omega}\left(\cos^2\omega H + \frac{\pi^2\zeta_h^2}{\omega^2}\sin^2\omega H\right)$$
$$\cos 2\pi\left(\frac{hx}{a} - \zeta_h H - \alpha\right) \qquad (3)$$

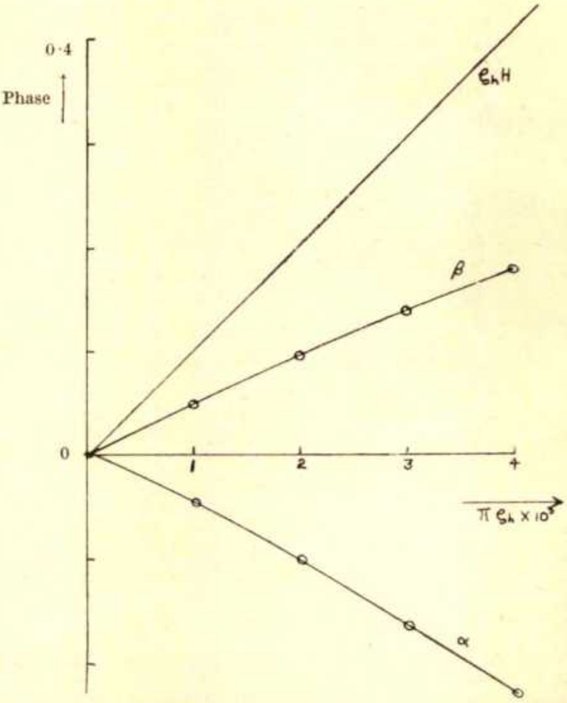

Fig. 2. Graph to show the relationships between α, β, $\zeta_h H$ and ζ_h for values of ζ_h up to $\frac{4}{\pi} \times 10^{-3}$. $F_h H = \pi/2$ and $H = 200\pi$ Å.

and then:

$$2\pi\alpha = \tan^{-1}\left\{\frac{\sigma E_h \cos\omega H - (\pi\zeta_h/\omega)\rho G_h \sin\omega H}{\rho G_h \cos\omega H + (\pi\zeta_h/\omega)\sigma E_h \sin\omega H}\right\} \qquad (4)$$

Of importance in equation (3) is the cosine term in x and the associated phase factor $\beta = (\zeta_h H + \alpha)$. The cosine term gives rise to the periodic undulations of intensity which are made visible when a microdensitometer, for example, is made to scan across several fringes. The phase term β, however, can in some circumstances lead to an anomalous effect, where the fringes lie at a considerable angle γ to the true lattice plane direction. In Fig. 1, the fringes at A make an angle of $\gamma \sim 8°$ with the parallel sides of the crystal. This effect can be predicted theoretically with the two-beam theory if representative values are chosen for H and F_h (see ref. 4*b*) and calculations of α and β made for specific values of excitation error ζ_h. Fig. 2 shows the results of such a calculation. The effect of adding the α curve to the $\zeta_h H$ line certainly leads to a β curve of reduced slope, but the graphs do not mutually cancel. The fact that the final curve has a finite slope implies a continuously changing phase factor as the excitation error increases to a value of $4/\pi \times 10^{-3}$ and, in this range, this must necessarily mean that the fringes are not parallel with the crystal sides.

It is to be concluded that, although fringe spacings in a perfect crystal are indicative of the actual separations of molecular planes[6], both the separation and the direction of fringes at or near a Bragg extinction contour must be treated with reserve.

I thank Dr. F. P. Bowden for help and encouragement, Drs. J. W. Menter, V. E. Cosslett and M. J. Whelan for discussion, and Imperial Chemical Industries, Ltd., for crystal specimens.

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Magnetic Energy

I WOULD like to comment, if rather tardily, on one paragraph of Prof. Ferraro's review[1] of my book, *Electromagnetism and Relativity*[2]. He says: "Prof. Cullwick appears to be under a misapprehension about the nature of the magnetic energy of a system of electric currents . . . we read that 'the hypothesis is introduced that the magnetic energy of a current circuit is the same as the kinetic energy of the effective conduction electrons, by virtue of their mean velocity along the wire'. The author then derives from this hypothesis a linear relation between the magnetic vector potential and the mean velocity of the charges . . . the hypothesis appears to be rather misleading. It is used in the chapter on superconductors to derive the familiar equations due to F. and H. London. Here the application is legitimate and indeed the linear relation mentioned above was first derived in connexion with the phenomenological theory of superconductors. But the relation cannot be universally true since it would seem to carry with it the implication that all conductors are superconductors".

From this, it would appear that Prof. Ferraro regards my hypothesis as leading to a macroscopic theory of superconductors identical with that of F. and H. London. This is not so. The hypothesis is that the magnetic energy of a quasi-steady conduction current is *in fact* the kinetic energy of all the conduction electrons which happen to be current carriers (that is, contributing to the macroscopic magnetic field) at a given instant, the effective electronic mass, m, including both the intrinsic mass and the mutual mass corresponding to the mutual electrokinetic energy of the electron with all the other effective conduction electrons in the circuit. The effective mass m will therefore depend on the configuration of the circuit, as does the coefficient of self-inductance, and will not have a constant value across the section of a finite conductor. If $\mathbf{A}$ is the component of the total vector potential due to the current in the single circuit considered, the hypothesis is, in general, summed up in the equation:

$$m\, v = e\, A_v \qquad (1)$$

so that in cases in which A and v are parallel:

$$m\, \mathbf{v} = e\, \mathbf{A} \qquad (2)$$

(In the first edition of my book equation (2) was used, incorrectly, in place of equation (1), which will be found in the second edition, p. 247.)

This is not part of the London theory of superconductors. The latter gives, for conductors large compared with the depth of penetration of the supercurrent, equality between the kinetic energy density of the supercurrent and the energy density of the magnetic field[3]; but these energies are regarded as separate and additive. For stationary conditions in an isolated singly connected superconductor, the London theory gives:

$$m\, \mathbf{v} = -e\, \mathbf{A} \qquad (3)$$

where $\mathbf{A}$ is the total vector potential and m the intrinsic electronic mass, which is not the same as equation (2).

In order to prevent further misunderstanding it is, I think, relevant to add some general comments.

(*a*) The magnetic energy of a current is a physical reality, not merely a mathematical fiction. Maxwell's general view of it has never been contested: "since we can form no conception of an electric current except as a kinetic phenomenon, its energy must be kinetic energy, that is to say, the energy which a moving body has in virtue of its motion. . . . We are therefore led to inquire whether there may not be some motion going on in the space outside the wire, which is not occupied by the electric current, but in which the electromagnetic effects of the current are manifested"[4a].

(*b*) Maxwell regarded magnetic energy as kinetic energy in the universal medium or æther: "According to our hypothesis, we assume the kinetic energy to exist wherever there is magnetic force, that is, in general, in every part of the field . . . and this energy exists in the form of some kind of motion of the matter in every portion of space"[4b].

(*c*) H. A. Lorentz's modification of Maxwell's theory, which when combined with the Lorentz transformation is universally accepted to-day, involves an immobile æther which is therefore incapable, by itself, of possessing kinetic energy. The theory retains, however, Maxwell's original assumption that an electric current *per se* has no inertia, an assumption since proved by experiment to be incorrect.

(*d*) One may then legitimately ask, of those who reject my hypothesis: "What *is* magnetic energy ?".

(*e*) Since an electron can be transformed, by collision with a positron, into electromagnetic radiation, it is reasonable to take its mass as being entirely electromagnetic, although as yet the only microscopic explanation of this is the unsatisfactory one in which classical theory is applied to the external field of a billiard-ball model.

(*f*) In modern theories of conduction the electron is treated in an over-simplified way. It is electrically charged, it is true, but its magnetic properties are largely ignored (an exception is L. Brillouin, *Helv. Phys. Acta*, **7** (Supp.), 47 (1934)). Its kinetic energy is somehow regarded as 'mechanical'. The logical deduction from this is that, when free electrons are accelerated in a vacuum to high energies, their kinetic energy eV is mechanical not magnetic, so that, contrary to all accepted theory, moving charges do not cause a magnetic field.

(*g*) If two electrons, macroscopically coincident, are moving in opposite directions with equal velocities, they cause no resultant macroscopic magnetic field. In a conductor carrying no current it is accepted, in modern theories, that the conduction electrons have random velocities with a Fermi-level of the order of 10^6 metres per sec. When an electric field is applied, almost all the conduction electrons continue to have paired velocities so that their macroscopic magnetic field is nil, but an extremely small proportion attain energies which are not balanced by electrons moving in the opposite direction. The macroscopic magnetic field must therefore be due to this very small proportion of electrons, moving with high velocities.

(*h*) The mechanism, possibly quantized, whereby an applied electric field imparts energy to the conduction electrons is unknown, and the hypothesis, based on a free electron gas, that every conduction electron has its energy changed by a small amount according to classical mechanics may well be entirely wrong. I have shown that if the magnetic field is due to a very small proportion of the available conduction electrons, moving with high velocities, their kinetic energy, even if m is taken to be equal to m_0, is of the same order of magnitude as the magnetic energy in typical current circuits.

(*i*) In my hypothesis the mass of a conduction electron is not to be considered as being limited to the 'particle'. It is the electron's share of the mass of the total electromagnetic energy, both internal and external, of all the current-carrying electrons in the circuit, and must be regarded as extending throughout the electromagnetic field. It thus satisfies Maxwell's general hypothesis quoted in (*a*) above. If the current is steady, this electromagnetic energy is considered to move with the same mean velocity as the electrons. There is no universal material medium through which electromagnetic energy can move only with the speed of light; but when the current changes, then the consequent disturbance of the steady conditions is communicated to a distant element of the field with the velocity of radiation.

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PROF. CULLWICK has directed attention to an incorrect statement, which I regret, in my review of the first edition of his book *Electromagnetism and Relativity*. As he says, the fundamental relation derived from his hypothesis (equation (1) of his communication) differs in sign from that usually adopted in the theory of superconductors so that Prof. Cullwick's hypothesis cannot be identified with the former—contrary to my statement.

My error, however in no way invalidates my contention that Prof. Cullwick's hypothesis is misleading. In the first instance, the relation $m\mathbf{v} = e\mathbf{A}$ is itself misleading, since it implies that the mean velocity of the conduction electrons is in all cases in the direction of the local vector potential. This is certainly not true, and in the second edition of his book the author has modified this relation in an attempt to overcome its shortcomings. Secondly, and this was my main contention, the hypothesis is misleading since in no sense can the magnetic energy be identified with the kinetic energy of the effective conduction electrons. In fact, the former is field energy propagated with the speed of light, and so cannot be identified with mechanical energy of the motion of the electrons. In any event, I cannot see what useful purpose such an identification would serve. In Maxwell's dynamical theory of electric currents a useful formalism is developed along the lines of Lagrange's analytical dynamics in which the magnetic energy of the electric current is shown to be the 'analogue' of the kinetic energy in a mechanical system. There is no implication that the two forms of energy are of the same nature. (In fact, Maxwell took care to call the energy of the currents 'electrokinetic' energy.) Any attempt to make it so appears to be a retrograde step, since most of the inertia associated with a system of currents is of electromagnetic origin. The artificiality of this hypothesis becomes, moreover, apparent when one considers that the definition of the inertia of the conduction electron would depend on the particular distribution of currents and on the position of the electron at any instant—surely a curious result.

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METALLURGY

Some Properties of Scandium Metal

SCANDIUM is one of the metals being used in an investigation into the alloying behaviour of plutonium with the metals of Group III which is being conducted at this Establishment. Since there is only limited published information on the properties of scandium itself, some experiments were undertaken on the metal. Recently, Spedding *et al.*[1] have reported some work with which the present results are in general agreement.

Thermal analysis, electrical resistance and X-ray diffraction techniques were employed for examination of the behaviour of the metal between room temperature and its melting point. The scandium was of reasonable purity, the main impurities being tantalum $\leqslant$ 0·1 wt. per cent, copper $\leqslant$ 200 p.p.m., silver $\leqslant$ 100 p.p.m., iron $\leqslant$ 20 p.p.m. and combined rare earths $\leqslant$ 100 p.p.m.

For thermal analysis measurements, a platinum-wound furnace was used and was filled with nitrogen at a pressure of 1/3 atm. to prevent volatilization of the scandium at high temperatures. A measuring platinum/platinum–rhodium thermocouple in a closed alumina sheath was in contact with approximately 1 gm. of scandium in a tantalum crucible. During initial runs, the metal when molten attacked the alumina, and a thin tantalum sheath was therefore placed over the end of the alumina for subsequent runs; at this point, the original scandium was replaced by a fresh specimen.

Thermal analysis arrests were invariably weak; but the observed melting point was cross-checked by noting the temperature at which the sheathed thermocouple could be pushed into the scandium during the initial run. The average value obtained for the melting point was 1,522 ± 5° C. However, an analytical check (Phillips, G., and Birks, F. T., private communication) showed that the sample had picked up an appreciable amount of tantalum, in agreement with an observation by Spedding[1]; nevertheless the value obtained does not differ greatly from that of 1,539 ± 2° C. observed by Spedding. A second arrest was observed at 1,373 ± 10° C., which may correspond to one observed at 1,335° C. by the American workers (Spedding, F. H., private communication). Owing to the high transition temperature, it was not possible to determine the crystal structure of the high-temperature modification with the available powder camera, but by analogy with certain of the rare earth elements it might be expected to be body-centred cubic[2].

The initial runs, in which an unprotected alumina sheath was used for the thermocouple, gave a value of about 1,490° C. for the melting point, suggesting that aluminium lowers the melting point of scandium.

Electrical resistance measurements were carried out on a short length of 0·040 in. diameter wire, which had been extruded using a pressure of 10 tons/sq. in. at 600° C. Current and potential leads were provided by spot welding 0·020 in. diameter platinum wires to the sample. The wire was then mounted in a vacuum furnace and the resistance recorded continuously as the temperature was raised; the method used has been described elsewhere[3].

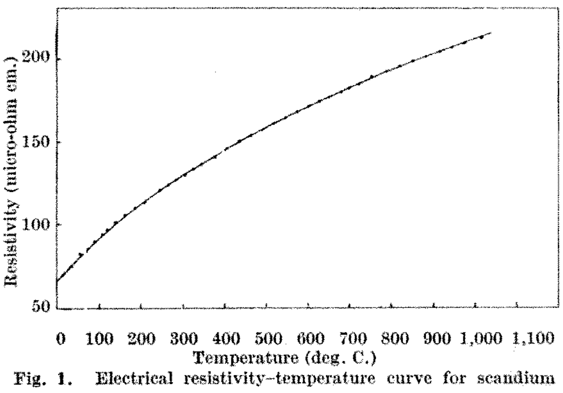

Fig. 1. Electrical resistivity–temperature curve for scandium

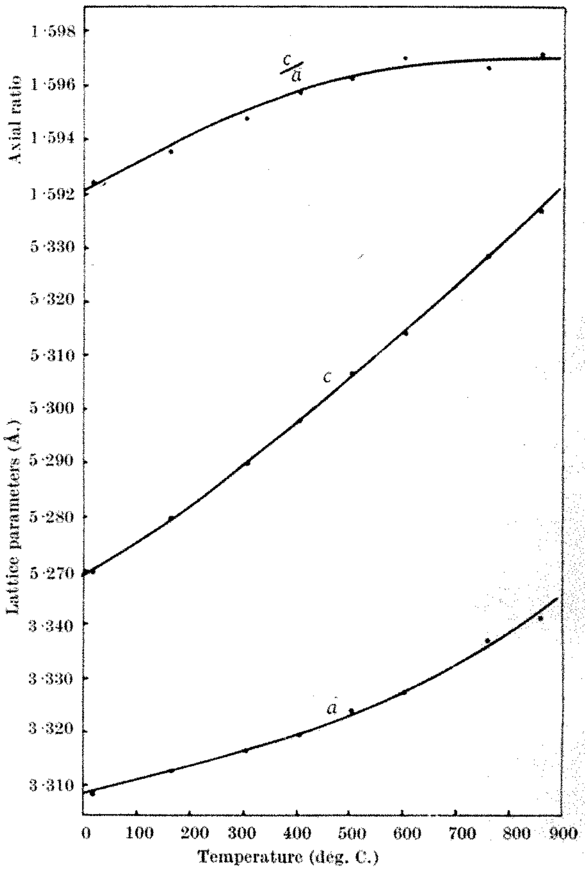

Fig. 2. Variation of scandium lattice parameters with temperature

During successive cycles measurements were taken to steadily higher temperatures; in the final cycle, the electrical contact failed at 1,160° C. On demounting the specimen it appeared that the failure was due to the formation of a low melting-point alloy at the contact points, implying that platinum markedly reduces the melting point of scandium. The resistance measurements at temperatures between 1,050° C. and 1,160° C. were variable, probably due to alloying, and the results shown in Fig. 1 have therefore been cut off at 1,050° C. There is no sign of any phase change taking place in the temperature-range 40–1,050° C., which is in agreement with the thermal analysis data. The 25° C. value of the resistivity was 72 micro-ohm cm., which is much greater than that of the common metals but is in reasonable accordance with the values for the rare earths, most of which lie in the range 55–75 micro-ohm cm. at 25° C.[2]. Previous investigators have given a figure of 66·6 micro-ohm cm. for the resistivity of scandium at 25° C.[1].

The temperature coefficient of resistivity decreases as the temperature is raised, the corresponding values being $3{\cdot}7 \times 10^{-3}$ at 100° C., $2{\cdot}1 \times 10^{-3}$ at 500° C., and $1{\cdot}3 \times 10^{-3}$ Ω/Ω −° C. at 900° C.

An attempt was made to determine the thermal conductivity of the wire using Kannaluik's method, but in practice the contact wires were not sufficiently massive to keep the temperature of the ends of the specimen down to ambient temperature as required by Kannaluik. An estimate can be made of the thermal conductivity by using the Weidemann–Franz relationship, since it is probable that the Lorenz ratio for scandium is similar to that of the early members of the rare earth series, that is, around $2{\cdot}7 \times 10^{-8}$ c.g.s. units. On this basis, the thermal conductivity of scandium would be 0·027 cal. cm./cm.^{2} sec. deg. C., at room temperature.

In the X-ray diffraction studies, using a 19-cm. high-temperature powder camera, scandium was examined at temperatures up to 850° C. The filings had been sealed in a thin-walled silica tube under vacuum but even so at a temperature of 750° C. the lines of scandium oxide appeared on the film. No attempt was made to take photographs above 850° C. because of the limitations of furnace power and the rapidly increasing strength of the oxide lines.

The structure remained hexagonal close-packed throughout the temperature range employed. The values of the lattice parameters and of the axial ratio are shown in Fig. 2; the behaviour is very similar to that of many of the rare earths[2], the c parameter increasing more rapidly than the a parameter at low temperatures, while at high temperatures the rate of increase of the parameters is comparable so that the axial ratio becomes approximately constant. A least-squares fitting of the experimental points gives the following values for the instantaneous coefficients of thermal expansion:

$$a = (5{\cdot}6_8 + 1{\cdot}3_7 t).10^{-8}/^\circ\,\mathrm{C}.$$

$$c = (12{\cdot}7_5 + 0{\cdot}5_7 t).10^{-6}/^\circ\,\mathrm{C}.$$

where t is the temperature (deg. C.). Thus the values for temperatures of 100° C., 500° C., and 800° C. are 7·1, 12·5 and $16{\cdot}6 \times 10^{-6}$/deg. C., for α_a and 13·3, 15·6 and $17{\cdot}3 \times 10^{-6}$/deg. C. for α_c.

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Control of Irradiation Embrittlement in Molybdenum

IRON, steel, and other body-centred cubic metals show an increase in ductile-brittle transition temperature as a result of neutron irradiation. In an attempt to elucidate the mechanism of this embrittlement for *EN*2 steel, Hull and Mogford[1] studied the effect of neutron irradiation on the parameters σ_i and k_y in the Petch relation:

$$\sigma_y = \sigma_i + k_y d^{-1/2} \qquad (1)$$

where σ_y is the yield stress and $2d$ the grain diameter. They found that σ_i, the lattice friction stress, increased, but k_y, which is a measure of the strength of dislocation locking, remained constant. They interpreted this to mean that the embrittlement was caused by point defects or groups of defects which impeded the movement of slip dislocations in their glide planes. This simple phenomenological description of irradiation embrittlement enables changes in ductile-brittle transition temperature caused by irradiation to be predicted from Cottrell's brittle fracture criterion[2]:

$$(\sigma_i d^{1/2} + k_y)k_y \geqslant \beta\mu\gamma \qquad (2)$$

once σ_i has been measured as a function of integrated neutron flux.

Johnson[3] has applied a similar method to that of Hull and Mogford in studying the irradiation embrittlement of molybdenum. His results showed that the embrittlement mechanism in molybdenum is more complex than for *EN*2 steel, and in interpreting his results Johnson suggested that in pure metals the point defects created during irradiation cause dislocation climb. It follows that the effect of irradiation on the mechanical properties of a pure metal such as molybdenum should depend on the density and distribution of dislocations in the metal before irradiation.

In an initial attempt to observe this expected dependence of irradiation embrittlement on dislocation density and distribution, experiments have been performed on molybdenum with two different types of dislocation structures. The first type of material was heavily cold-worked by swaging and the second type heavily cold-worked and then stress-relieved at 1,000° C. for ½ hr. to give a fine sub-structure. The starting material in each case was Murex sintered molybdenum containing nominally 50–100 p.p.m. carbon, 50 p.p.m. iron, 20 p.p.m. oxygen, 10 p.p.m. hydrogen, and 5 p.p.m. nitrogen. Both materials were machined into Hounsfield type tensile specimens and four batches of ten specimens of each were irradiated in *Bepo* for 3, 6, 9 and 12 months respectively at a temperature of 40–50° C. The corresponding integrated epithermal neutron fluxes were about 1, 2, 3 and 4×10^{19} n.v.t. The ductile-brittle transition temperatures of the unirradiated materials and of each of these eight batches of irradiated specimens were measured using a modified Hounsfield tensometer operating at a strain rate of $0{\cdot}88 \times 10^{-4}$ sec.$^{-1}$.

The ductile-brittle transition temperature of each batch was taken to be the highest temperature at which no appreciable plastic deformation occurred before fracture. On this criterion the transition temperature for both types of materials was $+ 120 \pm 10$° C. both before and after irradiation. Moreover, irradiation did not influence the shape of the load-elongation curve at any temperature. The transition temperature of recrystallized specimens of the same batch of molybdenum was $- 60 \pm 10$° C.

These results indicate that at least some measure of control of the irradiation embrittlement of body-centred cubic transition metals can be achieved by using materials with controlled dislocation structures. A fuller account and discussion of these results is to be published elsewhere.

This work was initiated under a U.S. Navy Department Contract and in its later stages was financed by the U.K. Atomic Energy Authority and Central Electricity Generating Board. It was carried out under the general direction of Prof. J. G. Ball.

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CHEMICAL ENGINEERING

Evidence of Phase Changes in Zirconia from Thermal Etching

ZIRCONIA is known to exist in at least two crystallographic forms. The stable form at room temperatures is monoclinic[1], but this transforms to a tetragonal lattice at some temperature between 950° and 1,250° C. (ref. 2), depending on the purity of the oxide. Cohn[3] has claimed a third form, hexagonal, obtained by heating for prolonged periods above 1,900° C. in a solar furnace. Up to the present, this has not been confirmed, but we believe we may have obtained evidence of three forms of zirconia.

We have recently completed the design, construction and testing of a carbon arc image furnace (unpublished work), which was used to investigate the melting of zirconia. Specimens were prepared from two batches of powder: one (prepared from Travancore beach deposits), with a range of particle size of 30–500μ, was claimed to be of very high purity except for 5·06 wt. per cent of hafnium oxide; the other (presented by Magnesium Elektron, Ltd.), with a range of particle-size of approximately 2–10μ, was analysed by the suppliers as containing, in terms of p.p.m. of zirconium content: 30 hafnium, < 20 manganese, 40 chromium, 460 iron, 153 silicon, < 30 vanadium, 42 nickel and 115 aluminium. The powders were compressed, without any lubricants or binders, into thin disks under a pressure of approximately 40 tons/sq. in., and melted in air in the arc image furnace. Melting took approximately 2 min., after which the specimens were allowed to cool. When the melted surfaces were examined under the microscope, grooves were seen at grain boundaries and some areas also showed surface striations the direction of which changed from one grain to another. These phenomena constitute thermal etching.

The conditions for and possible mechanisms of the formation of boundary grooves and surface striations have been discussed by Chalmers *et al.*[4]. It is concluded that a reduction in free energy of a strain-free lattice takes place by the reduction of surface and grain-boundary free energies. For ionic crystals, it has been shown by Lennard-Jones and Taylor[5] that the surface free energy depends upon the crystallographic orientation of the surface. Experimental evidence of this was obtained when single-crystal spheres of rock salt[6] and of alumina[7] were heated: crystal faces were developed and the spheres changed eventually to polyhedra.

Thermal etching, particularly of the grain-boundary grooves as opposed to the surface striations, has been used to determine the austenitic grain size of iron and steels[8]. This is possible because the grooves formed around the grains of the high-temperature phase persist after a phase change to the low-temperature form has occurred, so that two sets of grain boundary grooves are finally observed. The boundary grooves move as grain-growth occurs, without, in general, leaving traces of their former positions.

Although a number of investigations of the thermal etching of metals have been reported, little attention appears to have been given to the thermal etching of ionic crystals in general[9]. The results obtained with zirconia suggest that the method may be of general use as a rapid means of determining whether phase changes have occurred at high temperatures when the high-temperature phase cannot be retained by fast cooling. The accompanying photographs show part of the melted surface of zirconium with high hafnium oxide content (Fig. 1) and with low hafnium content (Fig. 2). Examination of Fig. 1 shows faint broad grooves delineating a small pentagonal region near the centre. A second set of narrower grooves meet in the centre of this region and in turn cross a third set of broad grooves which appear to be distinct from the first set. Some surface striations are also visible. In Fig. 2 grooves outline a large, roughly equi-axial, region in the centre. Within this region two sets of apparently distinct grooves meet, and a third groove, apparently distinct from both sets, crosses them. It is suggested that these three sets of grooves indicate that zirconia passes through three crystallographic forms in cooling from its melting point to room temperature. However, the possibility cannot be dismissed that some of the grain boundary grooves may be growth scars rather than 'phase scars'. But, the total heating and cooling time was relatively short—probably less than 5 min. for the cycle 1,000° C. to melting point ($\sim 2,700$° C.) to 1,000° C., whereas in the only instance where Chalmers *et al.* reported finding a growth scar (on a thermally etched silver specimen) the time at high temperature was 15 hr. By virtue of Cohn's claim and what appears to be a feasible interpretation of the evidence presented here, the behaviour of zirconia at high temperatures would seem worthy of further attention. It is, perhaps, significant that both Cohn and ourselves have used an image furnace in their preparation of the material. This method of heating[10] avoids contamination from furnace materials which may otherwise occur at high temperatures. Work on this topic is proceeding.

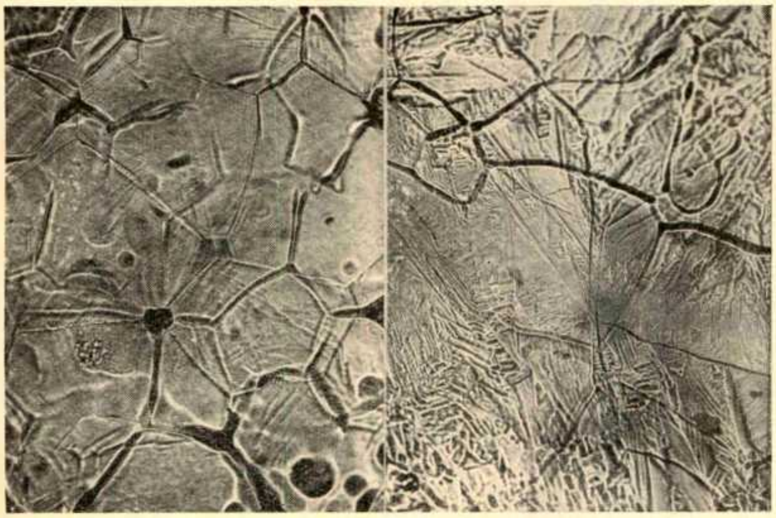

Fig. 1. High-hafnia zirconia. Fig. 2. Low-hafnia zirconia. (× 300)

We thank Prof. Frank Morton for the facilities he has made

available, our colleagues H. Holness[11] and R. W. Adams for the sample of Travancore zirconia and the determination of its hafnium oxide content, and Dr. S. J. Fletcher, of Magnesium Elektron, Ltd., for the sample of low-hafnia zirconia. One of us (G. W.) is indebted to the Department of Scientific and Industrial Research for a maintenance grant.

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CHEMISTRY

Gas Chromatographic Separation of Low-boiling Hydrocarbons using Active Alumina as Support for the Liquid Phase

SYSTEMATIC research into the behaviour of aluminium oxide as a support in gas chromatographic columns has, to our knowledge, been carried out by Hrapia[1] and Scott[2]. Hrapia was concerned principally with retention volumes and column performance and Scott with the deactivation of alumina with water.

We have found, at column temperatures between 0° and 70° C., that non-volatile liquids such as squalane, triethylene glycol and ββ′-oxydipropionitrile are suitable impregnating agents for alumina supports. The liquid phase is added to the support (γ-$Al(OH)_3$ containing 0·22 per cent by weight Na_2O, grain size 0·1–0·15 mm., calcined for 9 hr. at 400° C.) in dilution with a suitable volatile solvent (for squalane, petroleum ether, 40–70° C.; for polar liquids, methylene chloride) which is evaporated off prior to drying. During this final drying operation (5 hr. at 70° C.) alumina samples treated with polar liquids undergo losses of weight proportional to the quantity of liquid phase added; we can only explain these losses by assuming a release of chemically bound water from the surface of the alumina, suggesting the formation of surface compounds with the support. Aluminas impregnated with squalane show slight hygroscopicity even under drying conditions (compare Fig. 1).

Table 1

Liquid phase in alumina + liquid phase (per cent by weight)	HETP (mm.)		
	Squalane	ββ′-Oxydipropionitrile	Triethylene glycol
5	0·55	0·56	0·63
10	0·69	0·55	0·51
15	1·9	0·62	0·64
20–25	Column performance too low for the separation of low-boiling hydrocarbons		

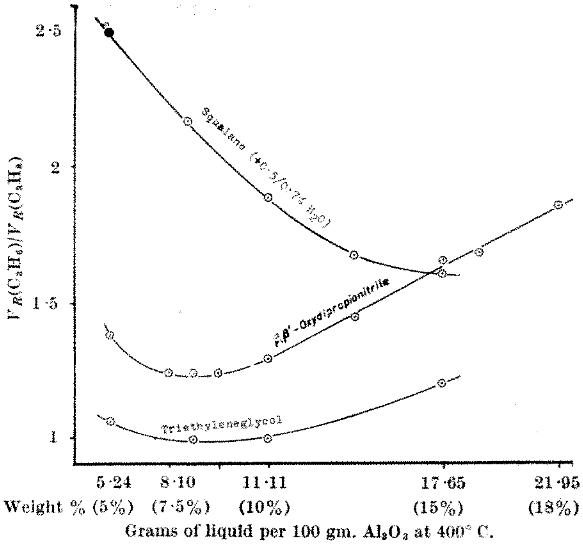

Fig. 1. Polarity charge with addition of liquid phase. V_R measured from air peak

It has been our experience that performances obtained with column packings having alumina as support are superior to those obtained with columns having an inactive support. The HETP values (height of equivalent theoretical plates) in Table 1 were obtained for packings of alumina + liquid phase

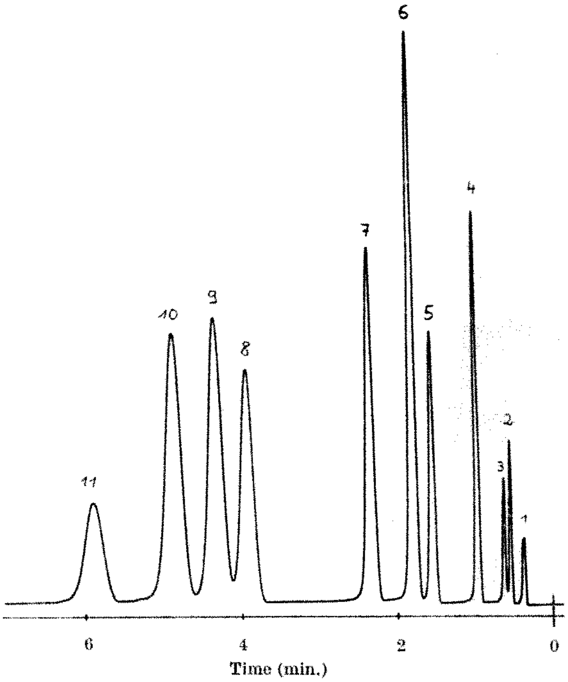

Fig. 2. Separation of C_1—C_4 hydrocarbons. (1) Air–methane; (2) ethane; (3) ethene; (4) propane; (5) propene; (6) *iso*-butane; (7) *n*-butane; (8) butene-1; (9) *iso*-butene; (10) *trans*-butene-2; (11) *cis*-butene-2

Sample size: 0·05 ml. (syringe). Column packing: 18 per cent by weight (= 20·95 gm. per 100 gm. Al_2O_3) of ββ′-oxydipropionitrile on 100–150 mesh Al_2O_3, calcined for 9 hr. at 400° C. Column dimensions: Copper tube, length 2 metres, inner diameter 4 mm. Column temperature: 0° C. Carrier gas: Hydrogen, inlet–outlet pressure ratio 2·66, inlet pressure 24·3 lb./sq. in. gauge, outlet flow-rate 70 ml./min. Thermal conductivity cell: heating current, 400 m.amp.; cell temperature, 0° C.

using *n*-butane as sample and hydrogen as carrier gas (outlet flow-rate 50–70 ml./min.).

Observations of the changes in retention volumes with change in the amount of liquid phase added show that retention volumes decrease continuously with increasing amounts of liquid phase (compared with Hrapia[1]).

Observations of the changes in polarity with change in the amount of liquid phase added are given in Fig. 1 in terms of the ratios of the retention volumes for propene and propane. With squalane the polarity of the alumina – liquid phase system decreases steadily with increasing amounts of liquid, whereas for polar liquids the polarity passes through a minimum. This latter observation indicates that to a certain extent the polarities of carrier and impregnating material compensate each other. The increase in polarity observed when adding polar liquids in amounts beyond those required for compensation is to be attributed mainly to the polarity of the surplus liquid. Mixed phases consisting of triethylene glycol and ββ′-oxydipropionitrile show polarities corresponding to the weight proportions of the individual compounds in the finished mixture.

The optimum conditions for gas chromatographic analysis, namely, short duration, column of low HETP, and polarity adequate for complete separation of sample components, are attained when all these considerations are taken into account. If the separation of air from methane is not required, the results tabulated with Fig. 2 are recommended for the analysis of C_1 to C_4 hydrocarbons (possibly including C_5). Under the same conditions, mixtures containing hydrocarbons up to C_7 have been separated with success. The reproducibility of the measurements is satisfactory. Further results and details will be published elsewhere.

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Gas Chromatographic Analysis of Permanent Gases using Standard Ionization Detector Equipment

Although the most common high-sensitivity detectors now in use for gas chromatography consist of either the flame[1] or the argon ionization device[2], such instruments cannot be used for the analysis of permanent gases when operated in the prescribed fashion. Thus in the argon chamber, the relatively low excitation potential of 11·6 eV. for metastable argon atoms is insufficient to ionize permanent gases which, unlike most organic compounds, have ionization potentials above this level. When we wished to employ gas chromatography for the analysis of gases extracted from the blood of man, it was decided to adapt existing equipment with as little alteration as possible. This was done by substituting ordinary commercial helium for argon as the carrier gas. The ionization chamber was a standard 1 cm. × 1 cm. microdetector which contained a 100-mc. tritium source.

Table 1. DETECTION OF PERMANENT GASES USING A STANDARD IONIZATION DETECTOR AND HELIUM AS A CARRIER GAS

Gas	Minimum sensitivity μl./sec.	Minimum sensitivity gm./sec.	Minimum sensitivity mole/sec.	Maximum linear range mole/sec.
Oxygen	0·015	2×10^{-8}	6×10^{-10}	$1{\cdot}6 \times 10^{-8}$
Nitrogen	0·035	4×10^{-8}	$1{\cdot}4 \times 10^{-9}$	$4{\cdot}2 \times 10^{-8}$
Hydrogen	0·040	$3{\cdot}6 \times 10^{-9}$	$1{\cdot}8 \times 10^{-9}$	$1{\cdot}8 \times 10^{-7}$
Nitric oxide	0·025	5×10^{-8}	1×10^{-9}	$2{\cdot}5 \times 10^{-8}$
Carbon monoxide	0·10	1×10^{-7}	4×10^{-9}	$6{\cdot}4 \times 10^{-8}$
Carbon dioxide	0·40	8×10^{-7}	2×10^{-8}	$1{\cdot}2 \times 10^{-7}$
Methane	0·10	7×10^{-8}	4×10^{-9}	$7{\cdot}2 \times 10^{-8}$
Ethylene	0·035	4×10^{-8}	$1{\cdot}5 \times 10^{-9}$	$4{\cdot}2 \times 10^{-8}$

Optimum operating conditions were obtained by selecting an applied potential which produced a standing current of 1×10^{-8} amp. at room temperature; usually 500 V. was sufficient. Contamination of the helium carrier stream by samples of permanent gases resulted in a decrease in standing current. The extent of this effect was taken as a measure of the quantity of gas being analysed. Although higher base currents gave an increase in sensitivity with a particular cell geometry, there was concomitant increase in the noise-level. Table 1 shows the minimum amount of the various gases which were detected under the conditions of the experiment. The detector was operated at 500 V. and the standing current was $0{\cdot}88 \times 10^{-8}$ amp. at 27° C. The noise-level was 5×10^{-11} amp.

The range of linearity for each gas is also listed in Table 1. By plotting a typical calibration curve as depicted for oxygen in Fig. 1, quantitative results can be extended beyond the linear range by at least a factor of five. Due to the mechanics of the detection system, the plot of decrease of current versus quantity of permanent gas passing through the detector tends to give a plateau at a minimum current-level. Although this range may differ somewhat for different gases and detectors of different design, the initial slope describing linear range is similar in all instances.

It should be noted that the ionization phenomena in operation in this application are different from those which would be expected if helium of extreme purity was used as the carrier gas[3]. Since increments in current were not noted, it was presumed that a reaction that would involve the contaminant gases and metastable helium atoms did not occur. At an applied potential of 500 V. to the detector, it is probable that the decreases in current which were

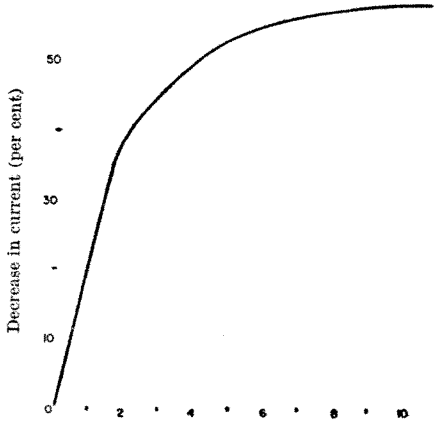

Fig. 1. Calibration curve for oxygen

observed were due to quenching of secondary electrons by molecules of the gas sample. The mode of operation of the detector also differs from that described by Willis[4], where the number of metastable argon atoms in the field were decreased by contaminating the argon carrier gas with ethylene. An electron mobility technique[5] in which a simple pulse generator is employed should offer considerable promise where additional sensitivity is required for the analysis of permanent gases. However, the sensitivity of the present method provides a very convenient compromise between this and conventional techniques where thermal conductivity detectors are used.

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α-Configuration of Fibrous Proteins

IT is now generally accepted that the polypeptide chain configuration in the group of fibrous proteins designated *k-m-e-f* by Astbury[1], which give the characteristic α X-ray diagram, is closely related to the α-helix described by Pauling, Corey and Branson[2]. In its simplest form, however, the α-helix does not account for the simultaneous appearance of the 5·2 and 1·5 Å. meridional arcs which are the diagnostic feature of the *k-m-e-f* group. Pauling and Corey[3] and Crick[4] suggested independently that these arcs might result from a systematic distortion of the α-helix in which the helix axis itself followed a helical path of large pitch. Detailed calculations by Crick[5] and Lang[6] showed that a group of three such helices twisted about a common axis to form a rope gives a satisfactory agreement with the observed diffraction pattern in the 1·5 and 5·0 Å. regions of the meridian.

We have recently investigated the diffraction pattern of the 3-strand rope in somewhat more detail using an optical diffractometer. While confirming the deductions of Crick and Lang, we find that the remainder of the meridional diffraction does not agree particularly well with the very wide-angle diffraction pattern that we have obtained from certain members of the *k-m-e-f* group. In Fig. 1 the optical transform of the 3-strand rope (*a*) is compared with the patterns obtained from α-keratin (*b*) and dried muscle (*c*). We have therefore explored the alternative possibility that in these proteins the α-helices, instead of being uniformly distorted, are composed of short straight segments with any distortion required for efficient packing being concentrated at a particular residue or residues and resulting in small but abrupt changes in the direction of the helix axis at these points. From the arguments put forward by Crick concerning the packing of helical polypeptide chains, groups of 2, 3 or more chains having the form envisaged above might be expected to form segmented ropes of similar average pitch to the uniformly distorted 3-strand rope.

The diffraction pattern of a segmented rope cannot be investigated without specifying the sequence of segment lengths and inclinations, but the general character of the transform is shown in Fig. 1*d*, which was obtained by an intensity addition of the diffraction from a number of segments 27 Å. in length inclined at a range of angles between 0 and 20° to the fibre axis. The maximum segment length is determined by the gradual divergence of the inclined helix axes and the accompanying departure from the idealized 'knob/hole' packing arrangement envisaged by Crick. A study of atomic models indicates that this length is probably in the region of 20–30 Å. The intensification of the eighth order meridional reflexion (Bragg spacing 24·6 Å.) that we have observed in the X-ray diagram of α-keratins after treatment with many heavy atom stains, for example,

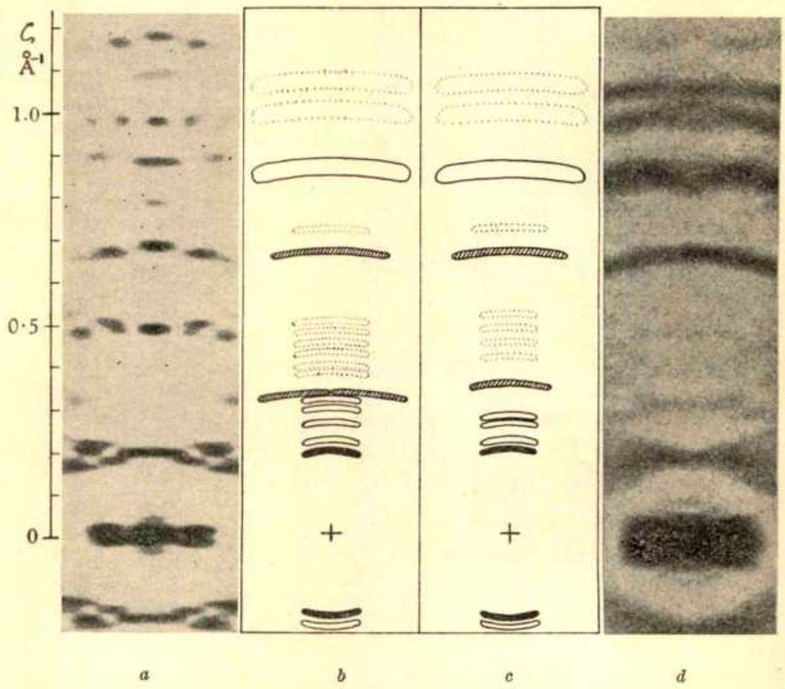

Fig. 1. *a*, The optical transform of the repeating unit of the three-strand rope described by Crick (ref. 5). The major helix is left-handed, the minor helix is right-handed and a β-carbon atom is included in the *L*-configuration about the α-carbon atom. *b*, The spacings and approximate relative intensities in the observed X-ray diffraction pattern from porcupine quill tip. Very weak reflexions are enclosed by a dotted line, weak reflexions by a full line, medium reflexions are shaded and strong reflexions shown solid. *c*, The observed X-ray diffraction pattern from dried toad sartorius muscle. *d*, The simulated optical transform from a segmented cable. The co-ordinate of the '5·2 Å.' arc is 0·19 Å.$^{-1}$ and that of the '1·5 Å.' arc is 0·67 Å.$^{-1}$

silver, mercury, copper and lead, may be associated with this length.

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BIOCHEMISTRY

Nature of Thyroxine-binding Globulin in Human Serum

The term thyroxine-binding globulin is often used to denote a serum protein fraction with a mobility intermediate between those of α_1- and α_2-globulins in paper or moving-boundary electrophoresis in veronal buffer, pH 8·6, ionic strength 0·05–0·10 (ref. 1). However, with the introduction of supporting media such as agar and starch gels and the use of different buffers, ionic strength or pH for paper electrophoresis, binding of thyroid hormones has also been detected in the 'prealbumin' zone[2]. Although the proteins of the 'prealbumin' zone obtained under different conditions are not likely to be identical, a strong thyroxine-binding property was detected in human serum prealbumin rich in tryptophan[3], as obtained from Prof. Schultze[4]. From chemical and immunological observations I concluded that the tryptophan-rich prealbumin, present as a complex in the α-globulin fraction, might be responsible for much of thyroxine-binding in human serum[5]. Since then, an α-globulin-type of thyroxine-binding protein has been obtained in this laboratory (unpublished results); the nature of this protein does not support the hypothesis that the tryptophan-rich prealbumin is involved in binding thyroxine. One possible explanation for this discrepancy is that Schultze's prealbumin preparation contained traces (1–2 per cent) of thyroxine-binding globulin which were responsible for the production of antibodies to it present in antisera against this prealbumin. In fact, multiple precipitation lines were observed in immunological studies using rabbit anti-human prealbumin serum[5]. The experiments to be described support this explanation and throw further light on the nature of the thyroxine-binding globulin in human serum.

A protein with strong thyroxine-binding properties was isolated (or prepared as an enriched fraction) from whole human serum, human cerebrospinal fluid and Cohn fractions IV-4 and IV-9 by a method which will be described elsewhere. The same major component was obtained from all the starting materials and will be referred to as thyroxine-binding globulin. A 1 per cent solution of this thyroxine-binding protein in 0·15 M sodium chloride was generally used for immunological studies. In Fig. 1 is shown a typical result observed when preparations of thyroxine-binding globulin were compared with tryptophan-rich prealbumin using rabbit anti-prealbumin serum (also obtained from Prof. Schultze) using Ouchterlony's double-diffusion technique[6]. Two major precipitation lines were seen for the tryptophan-rich prealbumin and Cohn fraction IV-9. A third line was also observed, if human serum was tested (not shown in Fig. 1). Only one line could, however, be detected in preparations of thyroxine-binding globulin, irrespective of the source. This precipitation line was continuous for the different preparations and with one of the lines obtained with tryptophan-rich prealbumin, Cohn fraction IV-9, and with whole serum, Cohn fraction IV-4 and cerebrospinal fluid, which were tested separately. This indicated that the thyroxine-binding globulin is an antigen common to all the preparations tested.

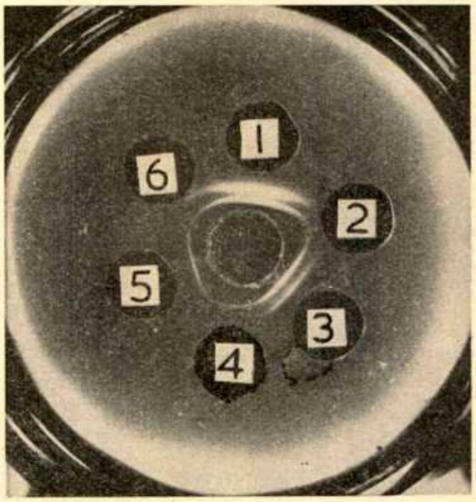

Fig. 1. Immunological interaction, using Ouchterlony's double-diffusion technique in agar gel, between rabbit antiserum against human serum tryptophan-rich prealbumin (ref. 4) (central well) and (1) tryptophan-rich prealbumin, (2) thyroxine-binding globulin prepared from unfractionated human serum, (3) Cohn fraction IV-9, (4) and (5) two different preparations of thyroxine-binding globulin made from Cohn fraction IV-9, and (6) thyroxine-binding globulin prepared from human cerebrospinal fluid;

Immuno-diffusion studies by the method of Allison and Humphrey[7] helped in deciding whether the tryptophan-rich prealbumin itself was not the common antigenic component. In this method the antigen and antibody troughs are placed at right angles, as shown in Fig. 2, and the angle θ formed between the precipitation line and the antigen trough is measured. The diffusion coefficients and consequently the relative molecular weights of the antigens were estimated from the equation, $\tan\theta = (D_g/D_b)^{1/2}$, where D_g and D_b are diffusion coefficients of the antigen and antibody respectively. Two major precipitation lines were again observed with the prealbumin (Fig. 2a), and the molecular weights of the two antigens were estimated to be about 60–65,000 for component 1 and 40–45,000 for component 2. Component 1 therefore corresponds to tryptophan-rich prealbumin itself, which has a molecular weight of 61,000 (ref. 4). With a preparation of thyroxine-binding globulin (Fig. 2b), only one line was observed, corresponding to an antigen of molecular weight of 40–45,000. This is the common antigen present in all the samples shown in Fig. 1; its lower molecular weight is compatible with the precipitation occurring nearest to the antibody trough in Fig. 1. It is therefore concluded that Schultze's tryptophan-rich prealbumin is not alone involved (either free or

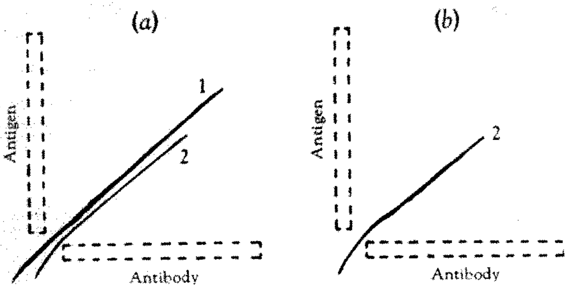

Fig. 2. Immunological reaction obtained using the technique of placing antigen and antibody troughs at right-angles, thus permitting an estimation of diffusion coefficients of antigens (ref. 7). The antibody trough contained rabbit antiserum against human serum tryptophan-rich prealbumin. (a) The antigen trough contained a 1·5 per cent solution of tryptophan-rich prealbumin. (b) The antigen was thyroxine-binding globulin obtained from Cohn fraction IV-9

complexed) in the transport of thyroid hormones in human blood, contrary to a previous suggestion[5], but that a distinct thyroxine-binding protein of a smaller size is responsible. The above immunological work also shows that the samples of tryptophan-rich prealbumin studied in this laboratory contained traces of thyroxine-binding globulin, estimated to be about 1–3 per cent of the protein from its unusually low binding capacity in the prealbumin samples.

Physical and chemical measurements made on preparations of thyroxine-binding protein have all confirmed the above conclusions. Zone electrophoresis on paper, cellulose acetate, agar gel and starch gel in various buffers, immunoelectrophoresis, sedimentation in the ultracentrifuge and measurement of tryptophan and sugar content reveal the differences between thyroxine-binding globulin and tryptophan-rich prealbumin. On the other hand, thyroxine-binding globulin resembles the α_1-glycoproteins of small molecular weight of Schultze and Schmid and their collaborators[8], although it may not be the same as the protein described by these workers. Some of the similarities and differences between thyroxine-binding globulin and other serum proteins, which will be described elsewhere, are summarized in Table 1.

Table 1. COMPARISON OF SOME PROPERTIES OF THYROXINE-BINDING GLOBULIN WITH THOSE OF SOME ISOLATED HUMAN SERUM PROTEINS

Sample	Electrophoretic mobility (-10^{-5} cm.^{2} V.$^{-1}$ sec.$^{-1}$)	Sedimentation constant ($S_{20,w}$)	Chemical analysis
TBG (unfractionated serum)	4·7 (*p*H 8·6) 1·7 (*p*H 4·5)	3·3	
TBG (isolated)	4·9 (*p*H 8·6) 1·8 (*p*H 4·5)	3·5	High sugar, low lipid, low tryptophan
α-Glycoprotein (ref. 8)	5·0 (*p*H 8·6)	2·9, 3·1, 3·5	High sugar
Prealbumin (ref. 4)	9·0 (*p*H 8·6)	4·2	High tryptophan

It is important to note that the sedimentation constant and electrophoretic mobilities of isolated thyroxine-binding globulin compare well with the values derived by Robbins, Petermann and Rall[9] for the major component in unfractionated human serum to which thyroid hormones are bound. The evidence presented here does not attempt to explain the diversity of thyroxine-binding protein zones when various conditions of electrophoresis of human serum are used[2]. It is concluded from these observations that the principal thyroxine-binding protein in human serum is an α_1-glycoprotein of small molecular weight; this necessitates a revision of the earlier suggestion that the tryptophan-rich prealbumin was alone involved in the transport of thyroid hormones in human blood[5].

I thank Dr. R. B. Pennell for Cohn fractions of human plasma; Prof. H. E. Schultze for human serum prealbumin rich in tryptophan, and immune rabbit serum; Dr. Marshall for human cerebrospinal fluid; Dr. K. Schmid for α_1-acid glycoprotein; Dr. P. Charlwood for ultra-centrifugal analysis and, finally, Drs. W. Gratzer and D. Tee for invaluable help and advice in zone electrophoresis, immunoelectrophoresis and tryptophan determinations.

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Influence of the Charge on a Serum Protein Molecule and its Ability to stimulate or inhibit the Phagocytosis of Bacteria by Leucocytes

THE present work was undertaken to investigate the effect of various human serum proteins upon the phagocytosis of *Staphylococci* by human leucocytes, and in particular to show the effect of modifying the net charge on the serum protein molecule upon the ability of the protein to stimulate or inhibit phagocytosis.

A modification of the slide-cell technique of Wright[1] has been used to assess the phagocytic ability of human leucocytes towards bacteria.

Human defibrinated blood from healthy Group O donors has been used. Part of any given sample of blood was used in its defibrinated condition without further treatment, but a part was centrifuged at high speed, and samples of serum and packed erythrocytes pipetted off under aseptic conditions and then re-mixed to give leucocyte-free blood of the same hæmatocrit as the original. The total leucocyte count of this latter sample was less than 3 per cent of the original count.

The slide-cells were constructed from two microscope slides separated by five strips of waxed paper, prepared under sterile conditions, in such a way that four shallow compartments were produced as shown in Fig. 1.

0·1 ml. of a 1 in 200,000 normal saline dilution of a peptone–water culture of *Staphylococcus aureus* (Wellcome Collection, No. *CN* 3638) was mixed with

0·1 ml. of an isotonic solution in saline of the protein to be tested, plus 0·8 ml. of the blood or leucocyte-free blood. The compartments of the slide-cell were filled as indicated and sealed around with melted paraffin wax, incubated at 37° C. for 15–20 hr., and then the number of bacterial colonies seen in ten low-power microscope fields noted for each compartment.

Human serum from healthy Group O donors was used for the preparation of the serum protein fractions. The serum was brought to 55 per cent saturation at 4° C. with ammonium sulphate (Analar grade), centrifuged at this temperature, and the precipitate re-dissolved in half the original volume of distilled water. Both this solution and the supernatant from the ammonium sulphate fractionation were then exhaustively dialysed against distilled water and afterwards lyophilized for storage.

The two serum protein fractions obtained were then used to prepare samples of both the acetylated and the methyl esterified derivatives, using the methods of Fraenkel-Conrat[2]. The methyl esterification was performed by treating a methanol solution, so far as it was soluble, of the protein at −15° C. with a small quantity of concentrated hydrochloric acid. Having kept the reaction mixture in the deep freeze for 48 hr., it was exhaustively dialysed against distilled water and lyophilized.

Electrophoretic separation of the proteins was done in duplicate on Whatman 3*MM* paper in barbitone buffer *p*H 8·6, ionic strength 0·05. After 18 hr. separation at 7 V./cm., one strip from each pair was dried and stained with bromophenol blue. The electropherograms were scanned in a slightly modified EEL scanner. The unstained strips were then eluted in order to measure ability of the protein fractions to stimulate or inhibit phagocytosis. The areas eluted are represented by the shaded areas in the electropherograms in Fig. 2. The eluant used was the same buffer as was used for the electrophoresis, and the eluted proteins were dialysed free of barbitone through 'Cellophane' tubing against normal saline containing 0·001 *M* phosphate buffer *p*H 7·2. The proteins were then concentrated by ultrafiltration through 'Cellophane' tubing to a final concentration of 5 per cent w/v, filtered free of bacteria, and used in the test of phagocytosis.

From Fig. 1 it is seen that the difference between the number of bacterial colonies which appear in a compartment containing leucocytes and in one which is devoid of leucocytes represents the antibacterial capacity of the leucocytes under the conditions in question. For simplicity this will be referred to as the phagocytic ability.

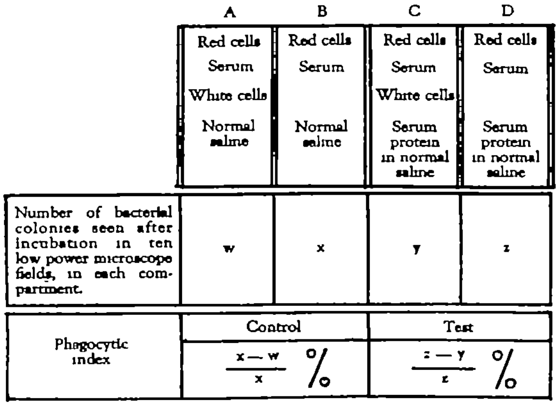

Fig. 1. Construction of slide-cells and calculation of results

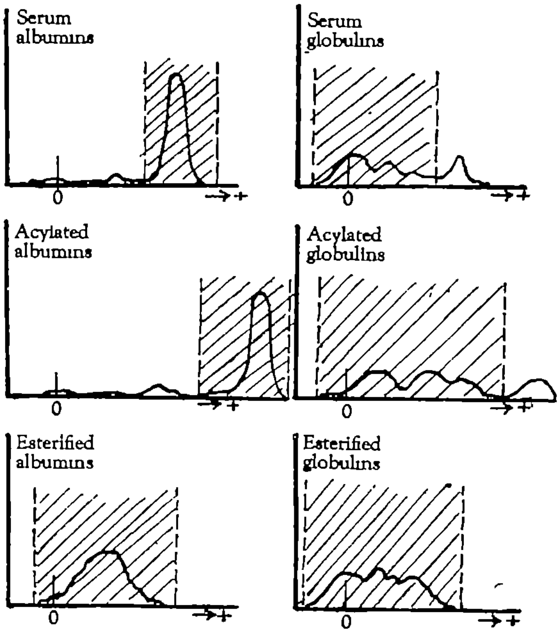

Fig. 2. Electropherograms of proteins, 0 being the point of application

Fig. 2 and Table 1 summarize the results obtained. In confirmation of the work of Tullis[3], the albumin fraction of the serum inhibits phagocytosis, and there is a significant stimulation with the globulins. Acetylation of a serum protein increases the negative charge on the molecule and either increases the inhibitory properties of albumins or decreases the stimulatory property of globulins. On the other hand, the esterification has been shown by electrophoresis to decrease the net negative charge on the serum protein molecule and increase the stimulatory effects of the globulins; but for some unexplained reason has had little effect on the properties of albumins in phagocytosis.

Table 1. PHAGOCYTIC INDICES IN A FINAL CONCENTRATION OF THE ADDED PROTEIN OF 1 PER CENT W/V

Substance	Phagocytic index	
	Mean	Standard error
Normal saline control	61	± 3·1
Serum albumins	43	± 2·7
Serum globulins	70	± 3·3
Acylated albumins	29	± 2·9
Acylated globulins	55	± 2·4
Esterified albumins	31	± 2·7
Esterified globulins	84	± 3·6

It would seem from these results that, for a given protein, the more negative its charge the more inhibitory it is towards phagocytosis, and conversely, the less the negative charge the more it will stimulate phagocytosis. In the case of the globulins, there seems to be the potentiality for either stimulation or inhibition of phagocytosis; yet the albumins seem to be capable only of inhibition. Clearly, therefore, there are other properties of a protein molecule besides its charge which determine its effect on phagocytosis.

I wish to thank Prof. G. A. H. Buttle for introducing me to the methods for measuring phagocytosis, and Dr. I. A. Hansen for suggesting the present investigation.

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Synthesis of Biologically Active Cellulose Derivatives of Enymes

Modified celluloses have been combined chemically with a variety of enzymes, with retention of enzymatic activity but with interesting and useful modifications of some properties. Derivatives have been prepared with carboxymethylcellulose and diazobenzylcellulose.

All derivatives are covalently linked and are stable to water, salt solution, acid, and buffer, and all can be dried by lyophilization without loss in activity. Derivatives of diazobenzylcellulose are insoluble, whereas those of carboxymethylcellulose vary from insoluble to water soluble, depending upon the characteristics of the carboxymethylcellulose. Soluble derivatives possess activity comparable with or greater than that of the free enzyme, but the insoluble derivatives are considerably less active.

Coupling with carboxymethylcellulose was effected by a modification of the azide method of Michael and Evers[1]. The entire process was carried out at 0° in a centrifuge bottle. Carboxymethylcellulose azide was prepared by suspending 1 gm. of its hydrazide in 150 ml. of 2 per cent hydrochloric acid solution and slowly stirring in 9 ml. of 3 per cent sodium nitrate solution. After 20 min., insoluble carboxymethylcellulose azide was separated by centrifugation and washed with 150 ml. of dioxane and then with distilled water to neutrality. Soluble carboxymethylcellulose azide, in the supernatant liquid, was precipitated by adding methanol to a final volume of 62 per cent, separated, and then washed several times with phosphoric acid solution at *p*H 2 before a final wash with distilled water. The carboxymethylcellulose azides were then separately stirred with 250–450 mgm. enzyme in 100 ml. of 0·05 *M* phosphate buffer at *p*H 8·7 for 2 hr. The insoluble derivative was then adjusted to *p*H 4 with 0·1 *M* phosphoric acid, separated, washed twice with 150 ml. of *p*H 3 water and once with distilled water, and finally lyophilized. The soluble derivative was precipitated at *p*H 3·5 with methanol (13 per cent) at − 5°, washed once with dilute phosphoric acid at *p*H 2 and once with water, and then lyophilized.

Soluble derivatives were obtained from more highly substituted cellulose, whereas lower degrees of substitution yielded derivatives with varying solubility properties. For example, a cellulose with low substitution (carboxymethylcellulose azide was prepared from carboxymethylcellulose products with different carboxyl equivalents obtained from Hercules Powder Co., Wilmington, Delaware, and Brown Co., Berlin, New Hampshire) yielded only an insoluble derivative, while one with an intermediate degree of substitution yielded an enzyme derivative that was soluble at *p*H 7 but insoluble at *p*H 3. It is not possible to be specific in predicting the solubility of the derivatives because of such factors as the uniformity of substitution, the number of free carboxyl groups in the final product, and the condition of the cellulose fibre.

Table 1. Comparison of Protein coupled and Enzyme Activity demonstrated by Chymotrypsin Derivatives (Typical Results)

Substitution on cellulose	Low	High		
Solubility of derivative	Insoluble	Insoluble	Alkali-soluble	Soluble
Mgm. protein coupled*/gm. derivative	95	350	221	558
Mgm. enzyme equivalent†/gm. derivative	30	86	172	1,071

* The protein was determined by spectrophotometric analysis at 280 mμ on the solutions before and after coupling.

† The quantity of enzyme in the derivative was calculated by comparison with the activity of the free enzyme on acetyl tyrosine ethyl ester (ref. 2).

Because of variations in the amount of carboxyl substitution on the cellulose, the quantity of enzyme coupled varies. The greater the original degree of substitution the greater the enzymatic activity of the derivatives; surprisingly, the activities of the soluble derivatives are equal to or greater than the activity of the free enzyme (Table 1).

In general, the activity of the derivative is related to the degree of hydration of the product rather than to the amount of enzyme coupled. This has been shown by comparing two insoluble derivatives with equal amounts of protein substituted on the cellulose. A derivative which had a tendency to swell in solution was 2–3 times as active as a derivative that did not show this property.

Limited studies indicated that both insoluble and soluble carboxymethylcellulose derivatives of chymotrypsin are more stable to heat than free chymotrypsin, the insoluble derivative possessing the greatest stability. There appears to be no shift in the *p*H optimum. The insoluble derivatives of trypsin and chymotrypsin are more active on small substrates, for example, acetyl tyrosine ethyl ester[2], than on large substrates, for example, hæmoglobin[3], showing some spatial limitation. Furthermore, the trypsin inhibitor of blood fails to inhibit the insoluble trypsin derivative, which further suggests spatial interference.

The second type of derivative was not so successful as the first, but proved that the cellulose–enzyme union is not restricted to one type of linkage. Insoluble diazobenzylcellulose derivatives of ribonuclease and chymotrypsin were synthesized by the coupling procedure of Campbell *et al.*[4]: 1 gm. of enzyme in 100 ml. of 0·05 *M* borate buffer at *p*H 8·7 was treated with 1 gm. of diazotized *p*-aminobenzylcellulose at 0°. Analysis indicated that the ribonuclease derivative was as much as 23 mgm. per cent protein, and the chymotrypsin derivative 34 mgm. per cent protein. However, the activities determined spectrophotometrically for ribonuclease[5] and titrimetrically for chymotrypsin[2] correspond to only 0·6 mgm. per cent, and 1·3 mgm. per cent enzyme, respectively. More work on this series may improve the activity incorporated into the derivative.

The use of modified cellulose in the synthesis of enzymatic derivatives should be applicable to an extensive series of purified enzymes. Not only may a study of these derivatives reveal information on how insoluble enzymes function, but it may also reveal a very practical way to use certain enzymes. For example, the insoluble carboxymethylcellulose-trypsin derivative has been used in our laboratory to effect a controlled chymotrypsinogen-to-chymotrypsin conversion. After the conversion, the derivative was easily recovered, leaving no trypsin contamination of the chymotrypsin; and with proper precautions, the derivative was re-usable. Similar applications could be suggested for other enzymes.

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Distribution of Coenzyme Q in Rat Liver Cell Fractions

COENZYME *Q* (Co*Q*), which is widely distributed in animal, plant and microbial sources, has been implicated in electron transport[1] and generally assumed to be associated with mitochondria. However, it has also been found in non-mitochondrial fractions of green leaves, although it appears to be concentrated in mitochondria[2]. A similar distribution has now been demonstrated in rat liver cell fractions.

Livers of stock colony rats were homogenized and fractionated by Schneider's method[3]. The following determinations were made: coenzyme *Q* by the decrease in absorption at 275 mμ on addition of sodium borohydride[4], succinic dehydrogenase by the reduction of dichlorophenol indophenol[5], $CoQH_2$ oxidation[6] (which is sensitive to antimycin and cyanide and therefore a measure of cytochrome oxidase), acid phosphatase[7] after pre-incubation for 1 hr. at 37° C., esterase activity with menadiol diacetate as substrate (Ramasarma, T., unpublished results), and protein by the biuret method[8].

Determinations of the distribution of coenzyme *Q* in rat liver cell fractions, giving a set of typical results of a number of experiments, are presented in Table 1. The mitochondrial fraction contained only 41 per cent of the total coenzyme *Q* and the rest of it was distributed in other fractions. The presence of coenzyme *Q* in all the fractions was confirmed by reverse phase paper chromatography. The large proportion of coenzyme *Q* present in the nuclear fraction (37·5 per cent) does not appear to be due to contamination of whole cells or mitochondria, since the acid phosphatase, $CoQH_2$ oxidation and esterase activities were low. But, since 30 per cent of the total succinic dehydrogenase was recovered in this fraction, the possibility of contamination by mitochondrial fragments cannot be excluded. The amount of coenzyme *Q* present in the fraction of sub-microscopic particles, the total as well as on the protein basis, was about one-third of that in the mitochondria, but the activities of succinic dehydrogenase and $CoQH_2$ oxidation in this fraction were several times lower in content and specific activity in comparison to mitochondria. Therefore the coenzyme *Q* in this fraction cannot be of mitochondrial origin, but appears to be partly constitutive and thus a wider role for coenzyme *Q* is suggestive. Whether the coenzyme *Q* present in sub-microscopic particles participates in the two oxidative enzyme systems—reduced diphosphopyridine nucleotide–cytochrome *c* reductase and the oxidation of *L*-gulono-γ-lactone—will be of interest, particularly in view of the observation of an involvement of a lipid co-factor in the latter system[9]. It is interesting to note that vitamin K, also a lipid-quinone, was found to be similarly distributed: nuclei 24 per cent, mitochondria 61 per cent, and sub-microscopic particles and supernatant 15 per cent, as tested by its biological activity[10].

Table 1. DISTRIBUTION OF COENZYME *Q* IN RAT LIVER CELL FRACTIONS

	Nuclei	Mito-chondria	Sub-microscopic particles	Super-natant
Coenzyme *Q* μmole/gm. protein	0·570	0 940	0 330	0 071
(per cent)	37 5	41	14 5	7
Succinate dehydrogenase activity	0·0095	0·0240	0·0040	0·0030
(per cent)	30	50	6	14
$CoQH_2$ oxidation activity	0·0007	0·0095	0 0008	0 0004
(per cent)	9	78	5	8
Acid phosphatase activity	0·0040	0·0340	0 0150	0·0035
(per cent)	10	56	21	13
Esterase activity	0 005	0 285	0·925	0·240
(per cent)	4	17	46	33
Protein (gm.)	0·425	0·280	0·228	0 630
(per cent)	27	18	14	41

The enzyme activities are expressed as μmoles substrate-change per min. per mgm. protein at 37° C. The total quantity present in all the four fractions is taken as 100 per cent.

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Conversion of DL-*m*-Tyrosine to Dopamine in the Rat

THE formation of catecholamines *in vivo* has been demonstrated using phenylalanine[1], tyrosine[2,3], 3,4-dihydroxyphenylalanine (dopa)[2,4], and 3,4-dihydroxyphenylserine[5] as precursors. *m*-Tyrosine (3-hydroxyphenylalanine) has been considered in this laboratory as another possible precursor, for it is decarboxylated by mammalian organs *in vitro*[6] and *in vivo*[7], and its decarboxylation product, *m*-tyramine, is found in normal[8] and phenylketonuric[9] urines, and in urine of subjects who have received an inhibitor of monoamineoxidase[10]. The recent characterization of leptodactyline as the trimethyl-ammonium salt of *m*-tyramine[11] suggests that at least some animal species can form *m*-tyrosine, although this amino-acid has not yet been identified in any natural source.

Table 1. URINARY EXCRETION OF DOPAMINE IN THE RAT

Exp.	Compound administered*	Dose (mgm./kgm.)	Mean-body-weight (gm.)	Dopamine excreted per rat (μgm.)	Recovery of *m*-tyrosine as dopamine (per cent)
1†	None		238	0·7	—
	DL-*m*-Tyrosine	100	228	38	0·26
	DL-*m*-Tyrosine	100	212	28	0·19
2	None		150	1·5	—
	DL-*m*-Tyrosine	83	148	51	0·47
	DL-3,4-Dopa	5	147	162	28·20
	DL-3,4-Dopa	15	172	449	22·50
3	DL-*m*-Tyrosine	50	218	22	0·24
4	DL-*m*-Tyrosine	100	101	25	0·27
	Same, but pre-treatment with reserpine (see text)	100	72	17	0·25

* Injected intraperitoneally in aqueous solution, controls received equivalent volume of saline. Three rats were caged together and their pooled urine was analysed for dopamine fluorometrically (ref. 13).

† In exp. 1 urine was collected for 5 hr.; in exps. 2 and 3, 19 hr.; in exp. 4, 21 hr.

We have found by administering DL-*m*-tyrosine (California Corp. for Biochemical Research, Los Angeles) to rats that excess dopamine (3,4-dihydroxyphenylethylamine) is excreted in the urine. In exps. 1–3 of Table 1, 0·19–0·47 per cent of the dose administered was excreted in the urine as the catecholamine. The urinary catechols obtained in exp. 1 were adsorbed on alumina and eluted with 0·5 *M* acetic acid; fluorescence of the eluate was measured at 335 mμ (activation at 280 mμ) in an Aminco–Bowman spectrophotofluorometer. With these conditions, dopamine fluoresces maximally, but specificity is lost by comparison with the oxidative procedure in which a fluorophore is formed from dopamine, but not from the other catecholamines, the catecholic acids[13], or *m*-tyrosine. This fluorescence, calculated as though it were all due to dopamine, gave a recovery of 0·26 per cent based on the DL-amino-acid; this is to be compared with the recovery of 0·19 per cent, estimated on the same material but using a specific fluorometric method[13]. In two other experiments of the same type the values for recovery were 0·27 and 0·28 per cent by the non-specific, and 0·25 and 0·27 per cent by the specific, fluorescence measurements, respectively. The yield from dopa is much greater (exp. 2).

Descending chromatography of the alumina eluates of exp. 1 was carried out on Whatman No. 3*MM* filter paper, using *n*-butanol/acetic acid/water (7 : 1 : 2), for 18 hr. After drying and spraying with ferricyanide reagent the eluate of urine of rats receiving *m*-tyrosine showed one spot; this was at the same position on the chromatogram as authentic dopamine. The eluate of control urine showed no such spot. *m*-Tyrosine is not adsorbed on alumina under the conditions used for preparation of the eluates.

Nine rats weighing 225–250 gm. were injected intraperitoneally with 200 mgm. of *m*-tyrosine per kgm. 30 min. later they were killed by guillotining, and the kidneys were removed for analysis. These organs contained 0·54 μgm. ± 0·09 (*S.E.*) of dopamine per pair of kidneys. A similar group of nine animals which had received 5 mgm./kgm. of tranylcypromine, a potent monoamine oxidase inhibitor, gave a mean of 1·98 μgm. ± 0·31 (*S.E.*). Control values (3 rats each) were 0·07 μgm. (range, 0·06–0·08) and 0·26 μgm. (0·17–0·30), respectively.

The excess urinary dopamine found after administration of *m*-tyrosine to rats as described could possibly be attributed to release of the amine from storage sites in the tissues. In order to verify this point, rats were injected intravenously with 10 mgm. of reserpine per kgm. to deplete the tissues of catecholamines, and 72 hr. later were given 50 mgm. *m*-tyrosine/kgm. intraperitoneally; controls were treated similarly except that the vehicle alone was substituted for the solution of reserpine injected. Matched groups receiving reserpine or vehicle, but not the amino-acid, were used to correct the values of the groups getting *m*-tyrosine; the level of this endogenous urinary dopamine is very low. It will be noted in Table 1, exp. 4, that the reserpine-treated and placebo rats excreted the same proportion of the administered amino-acid as dopamine. This indicates that a releasing action by *m*-tyrosine on 'bound' tissue dopamine does not play any part in the results obtained here.

The conversion of *m*-tyrosine to dopamine involves (1) oxidation of the ring in the *para* position and (2) decarboxylation. The order in which these occur and the mechanism of the ring oxidation are not known. Microsomal enzymes of the liver are known to oxidize aromatic rings in more than one position, but they do not appear to act in this way on L-phenylalanine[14]. Whether the same is true of *m*-tyrosine awaits further experiment.

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Reduction of Coenzyme Q by Succinic Acid Dehydrogenase

Crane, Hatefi, Lester and Widmer[1,2] have shown that coenzyme *Q* is reduced by mitochondria or derivative particles like electron-transporting particles, or succinic dehydrogenase complex, and that this reaction is sensitive to antimycin *A*. We have now succeeded in reducing coenzyme *Q* with a soluble preparation of succinic acid dehydrogenase, and this reaction was not sensitive to antimycin *A*.

Table 1. REDUCTION OF COENZYME *Q* BY SUCCINIC ACID DEHYDROGENASE

Assay conditions: 75 μM potassium phosphate, *p*H 7·2; 0·08 mgm. coenzyme *Q* in 0·02 ml. of anhydrous ethanol; 0·25 *M* sucrose to 1 ml. Incubation at 37°

Succinate (μM)	Enzyme (mgm.)	Duration of incubation (min.)	Inhibitor	Percentage of coenzyme *Q* reduced
7·5	0·1	10	None	20
7·5	0·2	10	,,	43
7·5	1·0	10	,,	86
7·5	0	10	,,	0
None	1·0	10	,,	0
7·5	0·45	2	,,	12
7·5	0·45	5	,,	18
7·5	0·45	10	,,	52
7·5	0·45	15	,,	77
7·5	0	15	,,	0
7·5	0·45	10	Antimycin *A* (30γ)	55
7·5	0·45	10	Antimycin *A* (60γ)	54
7·5	0·45	10	None	54
7·5	0·45	16	Antimycin *A* (30γ)	84
7·5	0·45	16	None	85

The enzyme was prepared from pig heart by the method of Neufeld *et al.* (preparation *B*, stage VI)[3]. The enzyme was dialysed at 0° against distilled water for 2 hr. The preparations showed succinic acid dehydrogenase activity, as measured by Slater's method[4], in the presence of methylene blue (12 µl. oxygen per mgm. of protein, per 60 min.), but did not exhibit any cytochrome *c* reductase and succinic oxidase activity. The reduction of coenzyme *Q* was determined by the method of Hatefi *et al.*[2]. The results are given in Table 1.

The results show that coenzyme *Q* is reduced by succinic acid dehydrogenase in the presence of succinate. Since this reaction is insensitive to antimycin *A*, it may be assumed that cytochrome *b* is not involved in this reaction.

A more detailed account of the experiments will be published in *Acta Biochimica Polonica*.

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PHYSIOLOGY

Nervous Activity in Relation to the Respiratory Cycle

IN the intact organism the physiological systems should show varying degrees of integration and interaction. Respiration is a cycle which appears likely to bear some relationship to moment-to-moment activity of the nervous system, because of its general biochemical effects, its wide afferent and efferent connexions, and its association with the brain stem reticular system. It is therefore relevant to ask whether changes in nervous activity occur preferentially in any part of the respiratory cycle. An answer can be sought in many facets of nervous function. In the electroencephalogram an abnormal feature very suitable for such study is the recurring, relatively stereotyped complex seen in sub-acute encephalitis[1] and possibly initiated from brain stem structures. A recent study in two cases[2] showed that these complexes arose preferentially at certain parts of the respiratory cycle; and the same obtained for eye blinks and voluntary movements. Further results reported here support the view that this asymmetrical distribution of activity is a more general phenomenon.

The object has been to determine the frequency of occurrence of a particular activity within each 1/10 of the respiratory cycle, a standard reference point on the activity being used for this analysis. The electroencephalogram, respiration and cardiogram were recorded simultaneously on 7, 8 or 16 channel apparatus in more than 40 patients undergoing neuropsychiatric investigation and in 12 normal adults. Respiration was measured unobtrusively with a thermocouple on the nose, and in some instances also by an expanding potentiometer on the chest. The respiratory ink traces showed distinct transition points at the onset of expiration, the interval between adjacent points being taken as a respiratory cycle. For analysis the respiratory tracing was obscured while standard reference points on the activity under study were marked on the trace. Each selected point was later projected on to its associated respiratory trace so that the 1/10 in which it fell could be determined, and frequency distributions compiled. The expiratory onset indicated by the thermocouple was probably not physiologically identical in all subjects or states of consciousness; but this seemed unlikely to produce misleadingly significant results. For statistical assessment the observed frequencies in the 10 intervals were compared (using chi square) with equal expectations at each interval. In each group subjects in which observation departed from expectation at the $< 0·01$ level were considered individually; the sum of the data from the rest of the group was assessed in the same way after a preliminary chi square analysis to ensure that the figures to be summed did not differ significantly.

'Spontaneous' arousal activity of the *K* complex type was studied in 16 subjects in quiet barbiturate-induced or natural sleep under ordinary laboratory conditions, analysis being restricted to those complexes arising without any provoking sound recorded or noted at the time. A masking background noise was used in some instances. The total frequency distribution for the onset of 1,700 complexes appears in Fig. 1*A*. There are two distinct patterns—(*A*) those with a peak in the first 1/10, and (*B*) the remainder; and as seen from the figure these differ essentially by 1/10 phase. Statistically, for 3 subjects with large numbers of arousals, $P < 0·01$; for the remainder summed, $P < 0·01$.

Spindle activity arising apparently spontaneously under the same conditions was studied in 16 subjects. The onset was again used as the reference point, analysis being restricted to activity occurring as a generalized change. In Fig. 1*B* appear distributions for the total (4,530), and for the two divisions (*A*, *B*) split as for arousal activity. The patterns are less striking. Statistically, in 3 subjects $P < 0·01$; and in the remainder summed $P < 0·02$.

Spike and sharp wave activity in barbiturate-induced sleep in temporal lobe epileptics was studied similarly, but the analysis was complicated by the

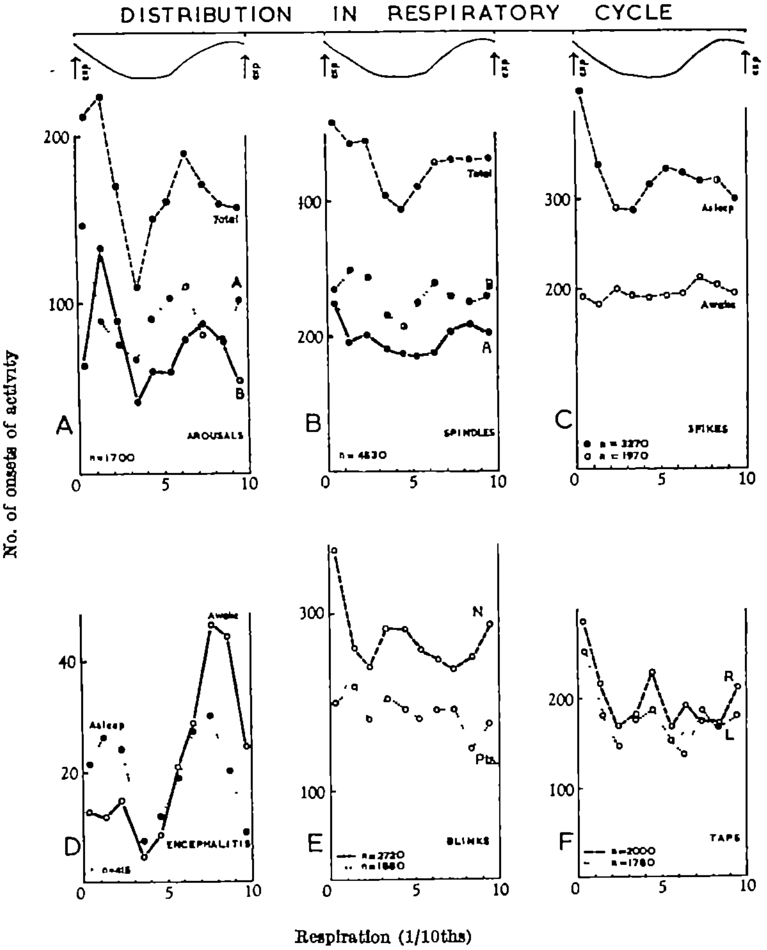

Fig. 1. Frequency of occurrence of events within each 1/10 of the respiratory cycle, taken as from the onset of one expiration (exp.) to the next. The events are the onset of: *A*, arousal activity; *B*, sleep spindles; *C*, spike/sharp wave activity; *D*, complexes in a case of sub-acute encephalitis; *E*, spontaneous blinking in normal adults (*N*) and patients (*Pts.*); *F*, voluntary tapping movements of the hands. (*n*, No. of observations. Total, 20,265)

tendency for the discharges to occur repetitively or in groups. Consistent results were obtained and the distribution of 3,270 discharges in 7 patients is seen in Fig. 1*C* ($P < 0{\cdot}001$). A tumour case provided a clear exception to this pattern. In the waking state there was no such uniform picture, as shown in Fig. 1*C* for 1,970 discharges in 7 patients.

In a case of sub-acute encephalitis the results obtained similarly for the onset of the complexes when awake and asleep are shown in Fig. 1*D* ($P < 0{\cdot}001$). Striking, though not identical, double-peak patterns have been seen in 2 other cases.

Spontaneous blinking was studied similarly in 8 normal adults unaware of the purpose of the investigation and free from visual distraction. Summed distribution for the onset of 2,720 blinks is shown in Fig. 1*E* (*N*). For 3 subjects $P < 0{\cdot}01$; for the summed remainder $P < 0{\cdot}02$. In 7 patients with more frequent blinking and some visual distractions the results were less uniform, as shown also in Fig. 1*E* (*Pts.*).

Voluntary repetitive tapping movements of the hand were studied similarly and the summed results for 10 subjects appear in Fig. 1*F* for right (*R*) and left (*L*) hands. The subjects were requested to tap every few seconds or so at will, preferably non-rhythmically, but without special attention; and the tapping was performed over long periods, interrupted by requests for pauses, changes of hand or frequency. All subjects were unaware of the purpose of the procedure and many that respiration was being recorded. For 2 subjects $P < 0{\cdot}01$; and for the remainder summed $P < 0{\cdot}001$. Fairly rapid rhythmic tapping in one demented and one defective patient showed only traces of this asymmetric distribution.

In natural sleep, dished or double-peak distributions were observed for the onset of slow activity in the presence of a tumour, and for slow activity leading on to spike discharges in focal epilepsy. Preliminary study of the suppression-burst activity of deep narcosis suggests an asymmetric distribution. Spike-and-wave type of epileptic activity showed in sleep a double-peak distribution which could be seen to some extent in the waking state, but not in all cases. A distribution similar to that for sub-acute encephalitis was seen in a lifelong epileptic with unusual recurring complexes associated with eyelid flutter. The double peak distribution may possibly be a basic pattern for some groups of activity. It might be noted that the respiratory distribution of heart beats (*Q* waves) has features in common with the above patterns when sinus arhythmia is present[1].

For any activity the distribution pattern depends on the frequency of repetition and the reference point chosen (changes in this latter producing a 'phase' or horizontal baseline shift). Nevertheless, patterns seen to a striking extent in sub-acute encephalitis are present to a lesser extent in other functions, though these are not necessarily independent of each other; for example, possibly the distributions of spike and spindle activity result from their association with arousal activity, which has much in common with encephalitic complexes. No methodological error has yet been found to account for these results. The possibility must be considered that the respiratory cycle reflects an influence which affects the probability of occurrence of many activities, an influence which, in the electroencephalogram, is best seen in sleep and certain pathological states where discharges may be initiated from brain stem structures. This influence, which need not be excitatory, might affect respiration or be secondary to it; and the reticular activating system would appear of first importance. It might impose a periodic tendency on otherwise sporadic activity, and influence the repetition interval or the likelihood of progression, for example, to a clinical attack. The termination and duration of activity are of interest in this type of study, which may assist the grouping of electroencephalogram phenomena.

Similar investigations of other aspects of nervous activity, for example, reflex and autonomic functions, are relevant.

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Changes in Mode of Respiration as an Indication of Level of Awareness

During a study of the peripheral autonomic electroencephalographic and psychological accompaniments of emotional states, it was discovered that frequently a change from thoracic to abdominal breathing, or vice versa, denoted more clearly than the electroencephalograph alone a change in the level of consciousness. The respiratory pattern was demonstrated by means of two expanding straps attached to potentiometer transducers, one around the abdomen and the other around the chest. The electroencephalograph, eye movements, galvanic skin resistance and peripheral vasomotor changes were also recorded, and there was a sound-level indicator.

In the upper example shown in Fig. 1 the subject is drowsy and snores are indicated. The mode of respiration is thoracic. Following a stimulus, the electroencephalograph shows arousal with a train of slow waves of high voltage. Accompanying this there is a change to abdominal breathing. In the lower part of the figure there is a shorter but similar arousal response and a change in the background activity of the electroencephalograph following a snore, but with a longer latency. The mode of respiration again changes to abdominal.

In Fig. 2*A* there is an apparently spontaneous arousal associated with an eye movement and a slight change to abdominal respiration. In Fig. 2*B*, following an external noise there is no arousal response in the electroencephalograph and no change in the background activity, but an eye movement initiates a clear change to abdominal breathing. In Fig. 2*C* there is again an apparently spontaneous arousal response in the electroencephalograph with no associated change in respiration, and to the right of the record a change in skin resistance (*PGR*) is followed by an electroencephalograph arousal and a change to abdominal respiration.

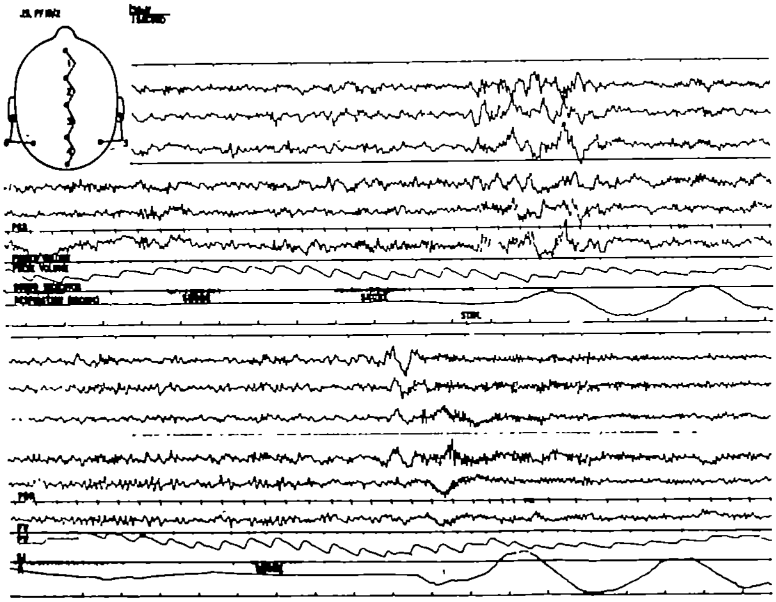

Fig. 1

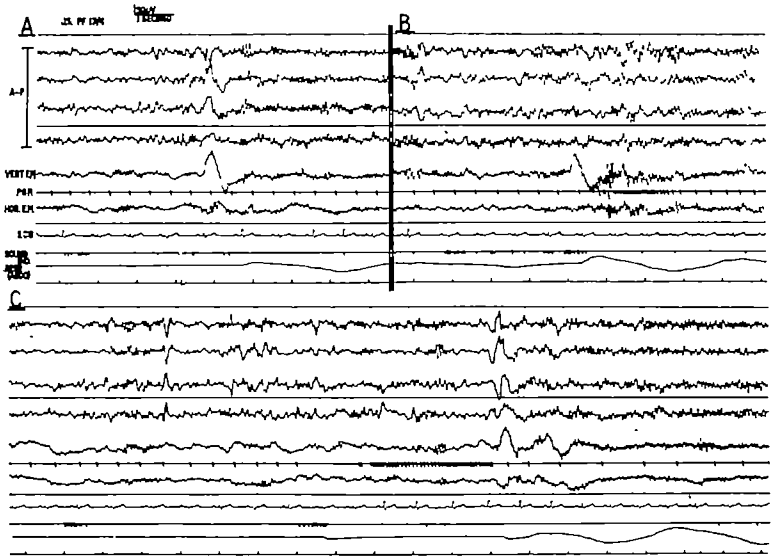

Fig. 2

A consistent change in mode of respiration during sleep as a response to a sound stimulus could be used as objective evidence as to the site of the lesion in deaf children, and may be of value in distinguishing deafness from mental deficiency. It is likely that studies of the arousal system in man might be furthered by close study of associated respiratory changes and also the problems of 'adaptation' and 'apperception' in sleep.

We have observed when using hypnosis that a change in the mode of respiration occurs as the subject becomes hypnotized and that this can be used as the first indication of a change in the state of the subject when there is no other objective evidence. It has been observed that snoring can occur with every clinical appearance of sleep on the suggestion that the subject go to sleep, while the electroencephalograph remains that of the waking state.

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The Synchronous Discharge of Apocrine Sweat Glands of the Welsh Mountain Sheep

Evidence that from time to time the apocrine sweat glands of the Welsh Mountain sheep discharge their contents synchronously over a large area of the fleece-covered skin has emerged from the observation that thermocouples attached to the skin anywhere beneath the fleece simultaneously register an occasional and brief rise in temperature which frequently exceeds both ambient temperature and deep body temperature as measured by a thermocouple implanted within the brachio-cephalic trunk[1] (Fig. 1).

The rise in skin temperature cannot be attributed to a change in tissue conductivity, but could be due to the generation of heat at, or near, the surface of the skin, as might occur if an aqueous fluid were abruptly discharged from the skin and absorbed by the fleece, since the uptake of water by wool, which is hygroscopic, involves an exothermic reaction[2-4]. As there is a rise in the humidity of air drawn continuously through a cup attached to a shaved area of skin simultaneously with the rise in skin temperature beneath the fleece elsewhere on the body surface (Fig. 1), while no rise in skin temperature is detected by a thermocouple attached to another shaved area of skin, this appears to be the explanation for the phenomenon.

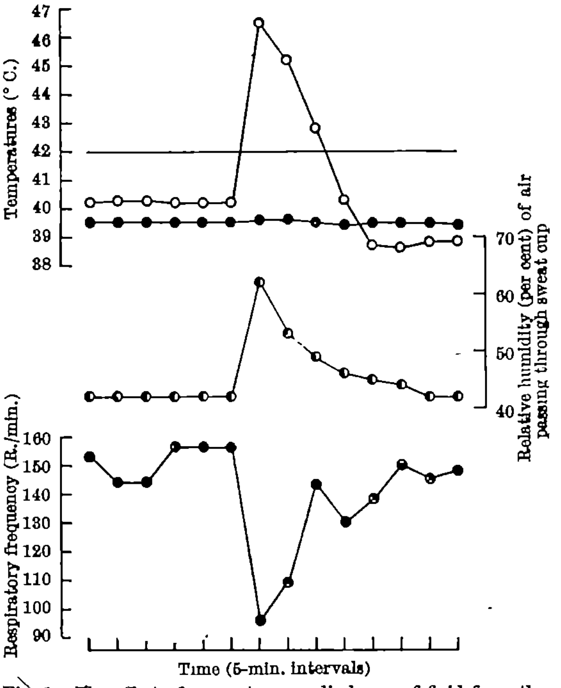

Fig. 1. The effect of a spontaneous discharge of fluid from the skin upon the skin temperature beneath the fleece (○), the relative humidity of air passing through a sweat cup (◐), respiratory frequency (○), and the temperature of the blood supply to the brain (●) at an ambient temperature of 42° C.

Table 1. Effect of Ambient Temperature on the Frequency of the Spontaneous Discharge of the Sweat Glands

Ambient temperature	Sheep No.	No. of 3-hr. exp.	No. of definite discharges	No. of possible discharges
20° C.	36 37 38	4 6 4	2 0 2	0 0 0
		14	4	0
42° C.	36 37 38	4 6 4	15 10 11	0 6 2
		14	36	8

The aqueous fluid is almost certainly derived from the apocrine sweat glands, of which there is one in association with each primary wool follicle[5], as it is known that the glands can be discharged by the administration of adrenaline (ref. 6 and Elliott, J. H., and Ferguson, K. A., personal communication), and the intravenous injection of 10–50 μgm. of adrenaline into a 25-kgm. sheep, under suitable conditions, will cause a similar rise in the temperature of the skin beneath the fleece. However, a second injection of adrenaline 30 min. later, or an injection of adrenaline shortly after a spontaneous discharge, may have only a small, or no, effect. This might be expected if the apocrine glands, following the expulsion of their contents by the contraction of the myoepithelium, are only slowly recharged by the secretory cells.

It is unlikely that the spontaneous discharge of the sweat glands results from a rise in blood adrenaline, as it is accompanied by a small depression (10–15 mm. mercury) in mean arterial blood pressure. A similar depression results from the intravenous injection of 3–5 μgm. adrenaline, but a larger quantity of adrenaline with a marked rise in blood pressure is generally necessary to discharge the sweat glands. The discharge stimulus may be mediated through a sympathetic innervation of the myoepithelium, as has been suggested for man[7].

The spontaneous discharge occurs with greater frequency at higher ambient temperatures (Table 1) but does not depend upon any rise in the temperature of the blood supply to the brain, and performs no obvious thermo-regulatory function. The Welsh Mountain sheep is generally able to maintain thermal equilibrium at an ambient temperature of 42° C. when evaporation is the only available channel of heat loss, whether or not there is a discharge of the sweat glands. Further, at high ambient temperatures, the mass discharge of the sweat glands is accompanied by a depression in respiratory frequency (Fig. 1) which more than compensates for any evaporation from the skin and may cause a small temporary rise in deep body temperature. A possible explanation for the greater frequency of discharges at higher ambient temperatures, which are accompanied by higher skin temperatures but not by a higher deep body temperature, is the local effect of heat on the secretory cells. An increase in the rate at which the secretion is formed would enable the glands to discharge more frequently.

A spontaneous mass discharge of the apocrine sweat glands followed by a quiescent period while the glands are being recharged does not appear to have been observed in other species with histologically similar glands. The ox responds to high ambient temperature[8], and the horse responds to exercise[9], with what appears to be a continuous discharge of sweat, which may be due to a continuous process of secretion, or to the asynchronous discharge of the glands. However, human apocrine glands become refractory to further stimulation after they have been discharged by injected adrenaline[7,10], and it has been suggested that there are synchronous variations in the sweat rate of human eccrine glands, which may be due to the simultaneous contraction of the myoepithelium[11].

It has been claimed that sweating is an important channel of heat loss in sheep when exposed to high ambient temperatures[12–14], and the possibility of a background activity apart from the intermittent mass discharge of the sweat glands remains. However, more recent work[15–17] has shown that panting provides an adequate channel for evaporative heat loss and that sweat gland activity may be of little importance in temperature regulation.

While this synchronous discharge observed in the sheep is not a characteristic of apocrine sweat glands, it may be a characteristic of the more primitive organ from which these and other skin glands have evolved. The skin glands of frogs and mammary glands both respond synchronously to humoral stimuli, and the sweat glands of the sheep, which may serve little or no thermo-regulatory function, may represent a relatively primitive type of apocrine gland.

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Influence of Calcium on the Potassium-Contracture of 'Slow' and 'Fast' Skeletal Muscle Fibres of the Frog

IT is well known that calcium is an important link in electromechanical coupling; however, there is little information about the underlying mechanism. Our knowledge about the action of calcium on the contracture of heart and skeletal muscles has been extended by a series of important observations[1–7]. Our experiments on electromechanical coupling have led us to investigate the action of calcium on tonic and twitch muscle fibres and to examine the problem whether the property 'slow' or 'fast' of a muscle fibre is influenced or caused by calcium.

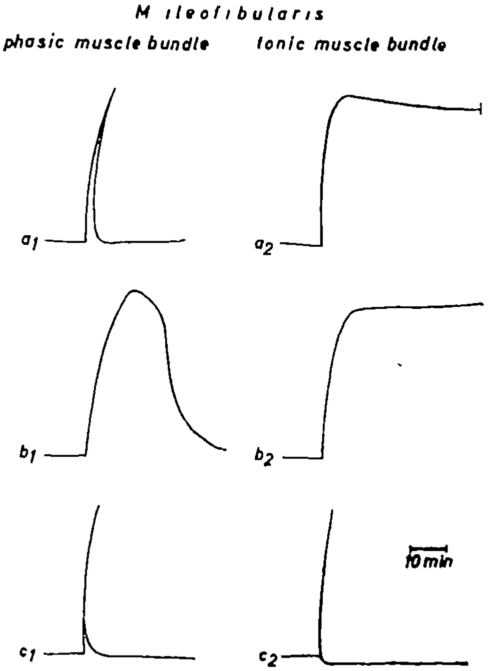

Fig. 1. Contraction (in isotonic potassium chloride solution) of fast (a_1 b_1 c_1) and slow (a_2 b_2 c_2) muscle-fibre bundles from m. ileofibularis of *Rana temporaria* after different preceding treatment (experiment from March 8, 1960). a_1 and a_2: fast and slow bundle after having been kept in normal Ringer's solution for 1 hr. before addition of potassium chloride. b_1 and b_2: these two bundles had been kept in isotonic, calcium-rich Ringer's solution for 1 hr. (88·0 m*M* sodium chloride; 2·5 m*M* potassium chloride; 18·0 m*M* calcium chloride). Potassium chloride solution in this case (93 m*M* potassium chloride, 18 m*M* calcium chloride). c_1 and c_2: These two bundles had been kept in isotonic, calcium-free Ringer's solution for 1 hr. (117·7 m*M* sodium chloride; 2·5 m*M* potassium chloride; 0·0 m*M* calcium chloride) before addition of potassium chloride

Fast and slow muscle fibre bundles from the m. ileofibularis (*Rana esculenta* and *R. temporaria*) were used for our experiments, the results of which are briefly discussed. Fig. 1a shows the typical reaction of a bundle of fast (a_1) and slow (a_2) muscle fibres to isotonic potassium chloride, that is, a rapid decline of the shortening of the twitch fibres and the prolonged contracture of the tonic ones.

Since the membrane potential is identical in both cases—it declines considerably and remains low—this means that there is a quick uncoupling of the process of contraction and depolarization in fast fibres, but this is not the case in slow fibres. Consequently there must be other governing factors. As is shown in the records reproduced in Fig. 1, this mechanism of coupling can be influenced by changing the calcium content of the Ringer's solution, which results in a more or less complete reversal of the specific function of either type of muscle fibre.

Curve b_1 shows the contracture due to potassium of a bundle of fast fibres after a preceding treatment with Ringer's solution of ten-fold calcium content. The reaction has become considerably more tonic. Even more pronounced is the effect of calcium on tonic fibres (curve b_2), which shows a higher and more prolonged contraction than that recorded in curve a_2. Finally, curves c_1 and c_2 show the effect of the absence of calcium on either type of muscle fibre: the contracture declines very rapidly.

This reversal of the function of fast and slow muscle fibres under the influence of varied calcium concentrations is not caused by a specific kind of membrane depolarization. It therefore seems to be the result of a direct action of the calcium on the mechanism of contraction.

The change in the contracting properties of fast and slow muscle fibres under the influence of varying calcium concentrations is not limited to contracture caused by potassium. It is also true for the so-called *Ermüdungskontraktur* and for the twitch. This will be discussed elsewhere.

The difference in the contractile properties of fast and slow muscle fibres suggests wide differences in the fundamental mechanism and ion kinetics.

Further investigations will be required to determine whether fast and slow muscle fibres have a different calcium content (probably through calcium pumps of different effectiveness), and whether the different resting potential (about 85 mV. for fast fibres; 60 mV. for slow fibres) inhibits the permeability of the tissue to calcium ions.

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Distribution and Fate of Rose Bengal

IT has been suggested[1] that complexity of the rose bengal plasma decay curve might be due to the uptake, by organs other than the liver, of different components of the dye. Biorck *et al.*[2] and Meurman[3], using commercial rose bengal labelled with iodine-131, obtained results which made this assumption improbable and have interpreted the plasma decay curve by assuming the existence of a reverse flow of dye from liver cells into the blood.

In order to investigate this problem further, a pure component of rose bengal (B.D.H.) was separated by paper chromatography according to Ishida *et al.*[4]. This pure compound has a mauve-pink colour and is not fluorescent; by ascending paper chromatography it has an R_F value of about 0·42 and by descending a value of about 0·47. Maximal absorption of a 0·002 per cent solution was at 548 mμ while for the total dye it occurred at 540 mμ. Labelling with iodine was performed, with slight modifications, according to Liebster and Andrysek[5]. The isolated component contained 41 per cent of the total radioactivity and no free iodine-131 was detectable.

Using this purified labelled rose bengal it was found that the plasma decay curve in the rabbit was precisely the same as when using the original multicomponent labelled rose bengal. This curve is approximately bi-exponential and closer examination shows that each of the two parts of the curve can be resolved into two exponential functions. The percentage disappearance-rates per min. in a typical curve had values of 28·9, 7·4, 1·2, and 0·11. It is concluded that the shape of the rose bengal plasma curve is not due to heterogeneity of the dye but solely to the biological situation.

Little information exists on extra hepatic uptake of rose bengal and no quantitative data are available[6–8]. The dye is bound by serum protein[9] and mostly by albumin[3]. Because trans-capillary passage of plasma protein takes place[10–13] it is likely that some extra-vascular diffusion of rose bengal will occur. It was found in normal rabbits that the amount of dye present in the extra-vascular space is not more than 3–4 per cent of the injected dose (60–80 μc. rose bengal labelled with iodine-131 in 5–10 mgm. carrier in 1–2 ml. of Ringer solution). This finding is supported also by the fact that in Meurman's data[3] about 6 per cent of the dose given is missing, and is most likely to be found in the carcass of the animal. By considering the size of the extra-vascular space[14], and the relative slowness of exchange[11], the later exponentials of the plasma decay curve appear to be explained.

Having to hand a single component of rose bengal it was thought worth while to see if the liver undertakes any biochemical conjugation of the dye as it does in the case of bromsulphalein[15]. Samples of rabbit bile, collected at different times following injection with labelled rose bengal, were subjected to ascending paper chromatography[4]. Only one radioactive spot with practically unmodified R_F value appeared for each sample, thus excluding transformation or conjugation of the dye. This agrees with the recent finding of Kubin *et al.*[16].

The fate of the albumin molecule after the remova of the rose bengal was also investigated. Rabbit albumin, prepared using carboxymethyl cellulose[17] and the elution gradient conditions indicated by Cohen[18], was labelled by the iodine monochloride method[19] with iodine-125 and then incubated with rose bengal labelled with iodine-131 (about 0·7

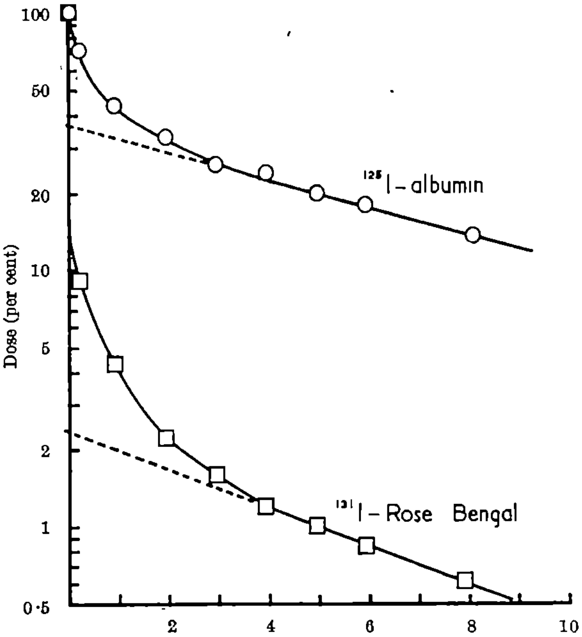

Fig. 1. Plasma radioactivities after injecting ^{131}I-rose bengal–^{125}I-albumin into rabbits

mole rose bengal per mole albumin). After dialysis at 2° C. against saline buffered with $0\cdot01\ M$ phosphate at pH $7\cdot35$, less than $0\cdot5$ per cent of the radioactivity was soluble in 10 per cent trichloracetic acid. The iodine-131-labelled rose bengal / iodine-125 labelled albumin complex was injected into two rabbits and radioactivities were measured in plasma, total body, urine and fæces. Fig. 1 shows that the iodine-131 label disappeared very rapidly from the circulation; it was found quantitatively in the fæces. The iodine-125 label, on the other hand, behaved exactly as it does when attached to normal rabbit albumin[20,21], that is, there was an initial disappearance of about half the albumin into the extra-vascular space and thereafter a steady exponential rate of loss occurred with half-lives of 130 and 140 hr. in the two experiments.

These experiments have demonstrated the lability of the dye–albumin complex and that the binding *in vitro* of rose bengal does not affect the catabolism of the albumin.

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HÆMATOLOGY

Inherited Hæmoglobin Ionographic Properties in Cotton Rats

A recent comparative study[1] showed that cotton rats (*Sigmodon hispidus*) were more variable, regarding hæmoglobin electromigration properties, than the other species examined. Several cotton rats of different hæmoglobin types were retained as breeders in order to investigate the genetic basis for these differences.

Hæmoglobin ionograms were obtained with Schleicher and Schuell 2043*A mgl* filter-paper strips in a Spinco Durrum type model *R* electrophoresis cell. The conditions of electrophoresis were as follows: $0\cdot0225\mu$ veronal buffer containing 15 per cent glycerol, pH 8 6, 20–26° C., 10 V./cm., 8 hr. duration. The dried strips were stained with bromphenol blue and were scanned in a Photovolt model 525 photoelectric densitometer.

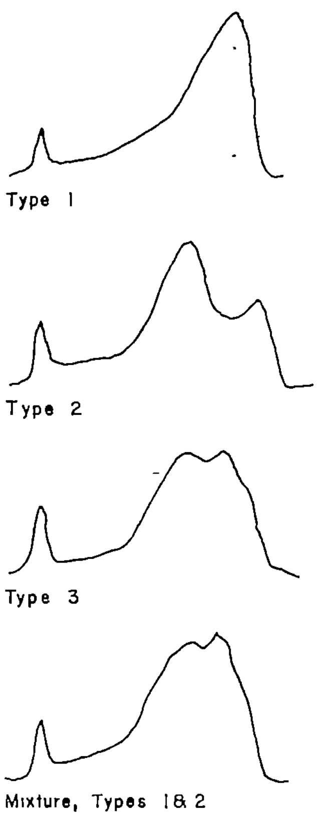

Fig. 1. Scanning curves of cotton rat hæmoglobin ionograms

Absorption scanning curves of the three different types of hæmoglobin ionograms found among cotton rats taken from wild populations are presented in Fig. 1. Among 23 cotton rats collected in Stanly County, North Carolina, 21 had hæmoglobin of type 2 and two had hæmoglobin of type 3. Of 11 cotton rats collected in Colquitt County, Georgia, seven had hæmoglobin of type 2, two had type 3, and two had type 1. Within the limits of resolution obtained, hæmoglobin type 1 seems to be a single component, but type 2 seems to contain two components, a major one plus a minor one of higher mobility. Type 3 is qualitatively indistinguishable, by this technique, from a 1 : 1 mixture of hæmoglobin types 1 and 2.

Protein that did not migrate from the origin is probably cellular debris that remained in the solutions after centrifugation. When hæmoglobin solutions were shaken with toluene or when centrifugation was prolonged these peaks did not appear; however, cotton rat hæmoglobins of all three types tended to precipitate from concentrated solutions, and omitting toluene extraction and shortening centrifugation time resulted in greater resolution.

In Table 1, cotton rat types *A*, *B* and *C* represent individuals that produce hæmoglobin types 1, 2 and 3, respectively. These results show that type *A* mated to type *B* produced only type *C* offspring. Type *C* rats mated to type *C* produced types *A*, *C* and *B*

Table 1. COTTON RAT MATINGS

Matings	No. of matings	Offspring			
		Type *A*	Type *B*	Type *C*	Total
B ♀♀ × *A* ♂	2	0	0	6 ♀♀ 2 ♂♂	8
C ♀ × *A* ♂♂	2	1 ♂	0	2 ♀♀ 2 ♂♂	5
C ♀♀ × *C* ♂♂	5	5 ♀♀	4 ♀♀ 3 ♂♂	6 ♀♀ 8 ♂♂	26
B ♀♀ × *C* ♂	2	0	4 ♀♀ 3 ♂♂	3 ♀♀ 2 ♂♂	12
					51

offspring in the approximate ratio 1 : 2 : 1, respectively. (A chi-square analysis of the actual ratio, 5 : 14 : 7, reveals that greater departures than this from the 1 : 2 : 1 ratio should occur by chance about 75 per cent of the time.) Type *A* mated to type *C* produced only types *A* and *C* offspring. Type *B* mated to type *C* produced only types *B* and *C* offspring. These findings plus the observation that 21 out of 23 rats taken at random in Stanly County, North Carolina, were type *B* (suggesting that type *B* cotton rats breed true) indicate that types *A* and *B* represent the homozygotes and type *C* the heterozygote of at least two contrasting genes. The fact that a 1 : 1 mixture of hæmoglobin from types *A* and *B* rats is almost indistinguishable from the hæmoglobin of type *C* rats suggests that these hæmoglobin genes act codominantly. The genes cannot be sex-linked, for heterozygotes may be of either sex. More data are needed, however, in order to ascertain whether the hæmoglobin locus in cotton rats is simple or complex.

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A New Inheritable Property of Human Sera : the InV Factor

Grubb and Laurell[1], Harboe and Lundevall[2] and Harboe[3] have described the serum groups Gma, Gmx and Gmb with the help of some rheumatoid-arthritic sera which were able to agglutinate O Rh+ red cells sensitized with an incomplete anti-D. Also with an anti-D rheumatoid-arthritic serum complex, Steinberg and Giles[4] have discovered a new factor (Gm-like) only present in the Negro sera but absent in the Whites. Ropartz *et al.*[5] have demonstrated that some sera withdrawn from healthy donors could be used for the Gm groups typings ; in fact, such sera sometimes possess a very pure anti-Gm specificity.

In particular, we studied one of these sera, Virm (the clinical examination shows no signs of arthritic diseases and the Waaler–Rose test is positive to 1/64). This serum, withdrawn from a blood donor, has the ability to agglutinate O Rh+ red cells, coated by an incomplete anti-D. We could demonstrate that : (1) some normal sera were able to inhibit its agglutinating power ; (2) this inhibitory power is inheritable and dominant in regard to the non-inhibitory one ; (3) this inhibitory property is contained in the γ-globulin fraction (Burnstein's method) ; (4) its relation with the Gma, Gmb and Gmx factors is actually studied.

This new serum group is temporarily called InV. It is found in 18·52 per cent of the sera of our population chosen from Seine-Maritime, France. Of 324 individuals tested, 60 were found to be InV(+). The inheritance of this factor has been confirmed by the study of 34 matings yielding 74 children. Out of this family material, 25 families with both parents InV(−) had children who were all InV(−). The anti-D used for the red cells coating had the phenotype InV(+). We have found people InV(−) and InV(+) whatever their phenotype regarding the Gma, Gmb and Gmx may be. Hence it seems that this new factor is independent of these other factors. But further research is necessary to disclose a possible linkage. Besides testing 399 Negroes from Dakar we have found 214 of them to be InV(+), that is to say, 53·63 per cent. Among these Negroes, the InV factor has been found when the individuals were Gm-like(+) as well as Gm-like(−). Therefore it looks as if the InV factor is also independent of the Gm-like factor described by Steinberg and Giles. This fact seems to us to be of profound importance, since until now the Negroes have appeared to possess Gm factors quite different from those of Whites. For example, all the Negroes have the phenotype : Gm(a+ b+ x−)[6,7]. Moreover, the Gm-like factor is present only among them. On the other hand, the InV factor is found in the Negro sera as well as in the White sera.

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Delayed Hypersensitivity to Homologous γ-Globulin in the Guinea Pig

THE existence in the rabbit of genetically determined antigenic differences ('allotypes') among homologous γ-globulins has been described by Oudin, who succeeded in preparing precipitating antisera against them after prolonged immunization with specific immune precipitates in adjuvants[1]. Considerable work has been done in an attempt to define the number of distinct serological groups and the number of genes controlling them in the rabbit[2]. The allotypic groups have been reported to be located on fragments I and II (as defined by R. R. Porter) of γ-globulin digested with papain[3].

Previous work on allotypes has been confined to the rabbit and to precipitating antibodies, with the exception of a statement by Oudin that he was able to observe some weak intraspecific precipitin reactions in guinea pigs[4]. In the present investigation, it is shown that guinea pigs, immunized with homologous γ-globulin in adjuvants, will rapidly develop a state of delayed hypersensitivity to the immunizing antigen, in the absence of antibody detectable by systemic anaphylaxis.

The experiments were carried out on white guinea pigs weighing 300–450 gm. In each experiment, animals from a single breeder were used which, although not inbred, were fairly homogeneous. γ-Globulin was isolated from the sera of several coloured adult guinea pigs labelled *A–D*. Various procedures were used for isolation—salt fractionation, starch electrophoresis, chromatography—to ensure that the immune reactions observed could be attributed to the γ-globulin component of the serum. The following antigens were used :

Preparation I : guinea pig γ-globulin prepared from the serum of a coloured guinea pig by batch chromatography, by Dr. D. R. Stanworth, University of Birmingham (unpublished work).

Preparation II : guinea pig γ-globulin prepared from the serum of a coloured guinea pig, *D*, by starch electrophoresis.

Preparation III : guinea pig albumin from the same serum as preparation II, by starch electrophoresis. Preparations II and III were kindly prepared for these experiments by Dr. E. Franklin, New York University.

Preparation IV : guinea pig γ-globulin prepared from mixed sera of coloured guinea pigs *B*, *C*, *D* and *E*, by repeated precipitation with 16 per cent sodium sulphate at room temperature.

Preparation V (1–9) : guinea pig γ-globulins prepared from sera of guinea pigs Nos. 17–25, by repeated precipitation with 16 per cent sodium sulphate.

Preparation VI (1–10) : heat-denatured guinea pig γ-globulin prepared by heating sera of guinea pigs Nos. 26–35 at 63° C. for 10 min. and dialysing against distilled water at *p*H 6 ; the precipitate being spun off and taken up in saline.

The antigens diluted in isotonic saline were mixed with an equal volume of complete Difco adjuvant containing 2 mgm. *M. butyricum* per ml. and emulsified. The antigens were injected in a volume of 0·2–0·5 ml. The animals received two immunizing injections of 0·5 mgm. or less of homologous γ-globulin 8–9 days apart in the foot pads. The intradermal test injections were made in the flanks, in a volume of 0·1 ml. The reactions were observed at 2, 4, 6 and 24 hr. All reactions had the appearance and timing characteristic of delayed sensitivity and measured 8–25 mm. in diameter. They were graded as described elsewhere[5].

A series of animals were immunized with their own isolated γ-globulin, native or heat-denatured, to serve as controls (exp. 4). The presence of antibodies was investigated by testing for systemic anaphylaxis.

Irrespective of the method of preparation, immunization of guinea pigs with homologous γ-globulin induces a state of delayed sensitivity which can be observed 10 days after a single injection, but becomes better established after two injections, still in the absence of the anaphylactic state. No reactions are observed with autologous γ-globulin. The ease and rapidity with which guinea pigs can be made to show delayed sensitivity to homologous γ-globulin contrasts with the long time required to raise allotypic antibodies in the rabbit, and may provide a useful approach to the study of the genetic control of allotypes.

Although no attempts were made to investigate the number of allotypic groups in guinea pig γ-globulins, experiment 3 suggests that there are a number of such groups in this species, as there are in the rabbit.

It is not surprising that allotypes, which are weak antigens, give rise in the guinea pig primarily to delayed sensitivity, since in this animal species delayed sensitivity is an earlier and more sensitive immune response than is the formation of serum antibodies, especially after a weak antigenic stimulus[5-7]. It is possible that in man, having the capacity to show easily delayed sensitivity, this type of immune reactivity to homologous or denatured autologous serum proteins may play an important part in disease as a consequence of chance immunization.

Table 1. SEVERITY OF DELAYED INTRADERMAL REACTIONS IN GUINEA PIGS IMMUNIZED WITH HOMOLOGOUS OR AUTOLOGOUS γ-GLOBULIN

Experiment 1. Albino guinea pigs immunized with 0·2 mgm. homologous γ-globulin, Preparation I

Animal No.	Day 10	Day 14	Day 21	Day 28
	40 γgm. Prep. 1	Boost	10 γgm. Prep. I	10 γgm. Prep. I
1	—		+ ±	+ + +
2	+ +		+ + +	+ + +
3	+ ±		+	+
4	+	100 γgm.	+ ±	+
5	+ ±		+ ±	+ ±
6	+ +	Prep. I	+	+ +
7	+ + +		+ + +	+ + +
8	+ + +		+ + +	+ + +

Experiment 2. Albino guinea pigs immunized and boosted (day 9) with 0 5 mgm. homologous γ-globulin preparation II. Animals tested with preparation II and also homologous albumin preparation III.

Animal No.	Day 21	Day 22	Day 27	
	50 γgm. Prep. II	2 mgm. Prep. II intravenously (for anaphylaxis)	50 γgm. Prep. II	50 γgm. Prep. III (albumin)
9	+ + +	– ve	+ + +	–
10	+	– ve	+ + +	–
11	+ +	– ve	+ + +	–
12	+ + +	– ve	+ + +	–
13	+ +	– ve	+ +	–
14	+ + +	– ve	–	–
15	+ + +	– ve	+ –	–
16	+ +	– ve	+ –	–

Experiment 3. 5 albino guinea pigs were immunized and boosted on day 8 with 0 5 mgm. of γ-globulin preparation IV (mixed γ-globulin from 4 guinea pigs *B*, *C*, *D* and *E*). They were tested on day 18 with 50 γgm. preparation IV and on day 20 with 1/50 dilution of the four individual sera *B*, *C*, *D* and *E*.

Animal No.	Prep. IV	Sera				Anaphylaxis, 2 mgm. Prep. IV : I-ven.
		B	*C*	*D*	*E*	
36	+	–	–	±	–	– ve
37	+ +	–	±	+	–	– ve
38	+ + ±	±	–	+ +	–	– ve
39	+ + ±	+ +	+ +	+ +	–	– ve
40	+ + +	+ +	+	+ + +	+	– ve

Experiment 4. 9 guinea pigs, Nos. 17–25, were immunized and boosted on day 9 with 0 5 mgm. of their own autologous γ-globulin (preparation V, 1–9). These animals were tested intradermally on day 21 with 50 γgm. of the same preparations. No positive reactions occurred.

10 guinea pigs were immunized and boosted on day 9 with 0 5 mgm. of their own autologous heat-denatured γ-globulin (preparation VI, 1–10). When tested intradermally with 50 γgm. of the same preparation, 9 were negative, one showed a positive (+) delayed reaction, suggesting that autologous globulin could become antigenic upon denaturation. More work is being done to investigate this possibility.

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RADIOBIOLOGY

Absorption of Inhaled Radioactive Water Vapour

THE local function of different regions of the lungs can be studied by the inhalation of various gases labelled with oxygen-15. Recently, water vapour has been labelled in this way. Oxygen-15 has a half-life of 2 min. and is produced by a continuous flow process by bombarding nitrogen with deuterons in the Medical Research Council cyclotron in the grounds of Hammersmith Hospital. Details of its preparation have been published[1]. The oxygen-15 is available in nitrogen containing 3 per cent oxygen as carrier at a flow of 0·45 litre per minute. Radioactive water vapour was prepared by adding 4 per cent hydrogen in nitrogen at a flow of 1 litre per minute and passing the resulting mixture over a palladium-type catalyst ('Catalyst *D*'; Baker Platinum Co.). The effluent contained 2 per cent of water as a vapour and had an activity of about 10 mc. per litre. The absence of unconverted oxygen was checked with a mass spectrometer.

Three normal volunteers inhaled two breaths of 1 litre each from a plastic bag connected to a respiratory valve box and mouth-piece. The activity in the lungs and respiratory tract was detected by two pairs of scintillation counters connected in coincidence. Good spatial resolution can be achieved with this arrangement, 95 per cent of the counts coming from a cylindrical core of tissue 4 cm. in diameter, between two opposed counters. Random coincidences were automatically subtracted. The counting methods have been described elsewhere[1].

When the first breath was taken, the counters were arranged over the peripheral lung fields so that the clearance-rate at the alveolar level could be measured, but we were surprised to find that almost no active gas reached the alveoli. For subsequent breaths, the counter-pairs were moved about so that the counting fields included the mouth, pharynx, trachea and peripheral lung fields. Table 1 shows the counting-rates (corrected for radioactive decay) after a typical breath. It can be seen that much of the activity was found in the mouth and mid-trachea, little penetrated to the carina, but virtually none could be detected in the peripheral lung field.

Table 1. COUNTING-RATES AFTER A SINGLE INSPIRATION OF RADIOACTIVE WATER VAPOUR

Site of counting field	Counting-rate (counts/sec.)
Mouth (A.P.)	170
Pharynx (lat.)	120
Upper trachea (lat.)	160
Mid trachea (lat.)	100
Mid trachea (A.P.)	110
Carina (A.P.)	33
Right lung base (A.P.)	3

* The opposed counters were either in front of and behind (A.P.), or on either side (lat.) of the subject.

The rate of removal of the activity from the mouth area after a breath of the gas was measured by fixing a pair of counters at that level for 2 min. During this period, the counting-rate fell by a half, and since this corresponds to the natural decay-rate, the removal-rate must be slow compared with this.

The rapid removal of inhaled radioactive water vapour by the upper respiratory tract is readily explained by exchange with the water lining the tract, but the efficiency of this process is remarkable. The results are in keeping with the observation that 98–99 per cent of inhaled tritiated water vapour is absorbed[2], since this high figure could only be achieved if the anatomical dead space was cleared of the labelled gas. In this respect, radioactive water vapour is perhaps unique, as other gases undergo little exchange in the dead space, hence its name. The results also fit in with the rapid warming and humidification of inspired air in the upper respiratory tract which have been described[3].

An interesting feature of the preparation of the water vapour was the large loss of activity which occurred when the gas was led through about 10 metres of 0·5-cm. bore polyvinylchloride tubing connecting the chemistry laboratory with the investigation room. Under these conditions, the yield was up to ten-fold less than when the gas was collected in a rubber bladder attached to the end of the catalyst tube. This loss of activity can probably be explained by exchange with water absorbed on the surface of the connecting tube in spite of attempts to remove any moisture present by passing dry air through the tubing. Water absorption on surfaces is a common problem in vacuum technology.

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Effect of Radioprotective Sulphydryl Compounds on the Oxygen Tension in the Spleen of Mice

IN a previous paper[1] the mechanism of the radioprotective action of the biologically active amines histamine, epinephrine, tryptamine and β-phenylethylamine was investigated. In studying the effect of these compounds on the oxygen tension in the spleen and bone marrow of unanæsthetized mice strong evidence was obtained that a relation exists between the percentage protection against a lethal dose of X-rays afforded by these compounds and the degree of hypoxia caused in the blood-forming organs. Furthermore, it was shown[2] that cyanide also causes hypoxia in the spleen as a consequence of vasoconstriction and that this effect may well explain its protective action in mice.

In the present communication experiments are presented on the effect of a number of protective sulphydryl compounds on the oxygen tension in the spleen and bone marrow of unanæsthetized mice and rats.

The experiments were performed on groups of female *CBA/Rij* mice weighing 18–20 gm. and on male *WAG* rats of 180–200 gm.

Table 1. CHANGES IN OXYGEN TENSION IN THE SPLEEN AND BONE MARROW RESPECTIVELY OF UNANÆSTHETIZED FEMALE *CBA/Rij* MICE, 18–20 GM., AND MALE *WAG* RATS, 180–200 GM., CAUSED BY A NUMBER OF PROPHYLACTICS AGAINST RADIATION. NEUTRALIZED SOLUTIONS OF ALL COMPOUNDS WERE INJECTED INTRAPERITONEALLY IN A VOLUME OF 0·5 ML.

Species	Organ	Compound	No. of animals	No. of animals in each specified class				
				l.i.	*s.i.*	*n.s.*	*s.d.*	*l.d.*
Mouse	spleen	3 mgm. cysteamine	18	6	4	2	6	—
	femur	3 ,, ,,	4	1	1	—	1	1 ?
	spleen	5 mgm. cystamine*	10	—	—	—	1	7 + 2 ?
	spleen	5 ,, ,, †	6	—	2	2	1	1
	spleen	7 ,, ,, †	4	—	—	—	1	3 (2 died)
	spleen	26 2 mgm. cysteine	6	1	2	1	2	—
	spleen	50 ,, ,,	11	3	3	1	2	2
	femur	50 ,, ,,	4	2	1	—	1	—
	spleen	75 ,, ,,	1	—	—	—	—	1 (died)
	spleen	3 mgm. AET‡	5	4	—	—	1	—
	spleen	5 ,, ,,	7	5	2	—	—	—
Rat	spleen	10 mgm. cysteamine	6	2	4	—	—	—
	spleen	20 ,, ,,	11	5 + 2[1] + 1 ?	1	1[2]	—	1
	spleen	30 ,, ,,	2	1	—	1	—	—
	femur	100 mgm. cysteine	2	1	1	—	—	—
	spleen	200 ,, ,,	6	5	—	—	—	1
	femur	200 ,, ,,	2	2	—	—	—	—
	femur	300 ,, ,,	3	1	1	—	1	—
	humerus	300 ,, ,,	6	4	—	—	1[2]	1
	spleen	400 ,, ,,	6	—	1[4]	—	1[3]	3 + 1[2]
	spleen	500 ,, ,,	2	—	—	—	—	2
	spleen	600 ,, ,,	1	—	—	—	—	1 (died)

* Manufactured by Labaz, Bruxelles.

† Manufactured by California Foundation for Biochemical Research, Los Angeles.

‡ Amino-ethyl-*iso*thiuronium bromide.

? After *l.i.* or *l.d.* means that this condition was present for a short time only to change soon to *s.i.* or *s.d.*, respectively.

[1] Fairly soon followed by *s.d.* or *l.d.*

[2] After a considerable time *l.i.*

[3] Fairly rapidly thereafter *l.i.*

[4] Followed by *s.d.*

The technique of the polarographic estimation of the oxygen tension in the spleen has been described elsewhere[1]. In addition the oxygen tension in the bone marrow of the femur in mice and of the femur and humerus in rats was estimated by a similar method.

The results were found to vary considerably within each experimental group. The different types of response obtained after injection of the protective compounds were divided so far as possible in five classes as follows: *n.s.*, either no effect or a rise or fall not exceeding 10 per cent of the pre-injection value; *s.i.*, a slight increase in oxygen tension between 10 and 40 per cent; *l.i.*, a larger increase exceeding 40 per cent; *s.d.*, a slight decrease between 10 and 40 per cent; *l.d.*, a larger decrease exceeding 40 per cent.

The results of the experiments are represented in Table 1, in which the number of animals belonging to each class is indicated for a number of protective compounds used in doses which were found to protect the animals against a lethal dose of total body irradiation with X-rays. In a number of cases the classification failed, which is indicated in Table 1.

Table 1 shows that in contrast to the effect observed after both the biologically active amines and potassium cyanide the effect of the sulphydryl compounds is subject to considerable variation. However, for most of the compounds studied the oxygen tension shows a tendency to rise rather than to fall. Cysteine at higher (just sub-lethal) doses only causes a decrease in oxygen tension.

A possible exception is cystamine, to which most of the animals react with hypoxia. This is in accordance with the view[3] that cystamine, being devoid of a free sulphydryl group, acts like a biologically active amine. The fact that amino-ethyl*iso*thiuronium bromide behaves like the sulphydryl compounds is consistent with the view that it is converted into mercapto-ethyl-guanidine[4].

In summary, these results strongly indicate that the protection afforded to mice by the sulphydryl compounds is not mediated by hypoxia of the blood-forming organs. Therefore their mechanism of action differs from that of the biologically active amines and cyanide.

A number of investigators have expressed the view that sulphydryl compounds have a protective effect other than the production of anoxia. The most recent observations are the experiments of Lothe and Devik[5] on the rabbit ear, those of Kohn and Gunter[6,7] on *Escherichia coli*, of Grant and Vos[8] on isolated rat thymocytes and of Vergroesen *et. al.*[9] on HeLa cells in tissue culture.

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Changes in Radioresistance of Sporulating Cells of *Bacillus cereus*

THE spores of bacilli represent a unique form of living matter capable of surviving many harmful factors of environment and of restoring intense metabolic activity under suitable conditions. The study of individual mechanisms of resistance of spores was directed mainly to the differences in the content of individual constituents of cytoplasm of spores and of vegetative cells.

One of the typical differences between vegetative forms and spores is the different cystine content. Hot trichloracetic precipitate of spores of some bacilli which have been studied contains about 4–5 times as much as this amino-acid as the vegetative cells[1–3]. On the basis of incorporation of cysteine labelled with sulphur-35 and calcium chloride labelled with calcium-45 during sporulation of *Bacillus cereus* on liquid medium, it was observed that the maximum incorporation of sulphur-35 appears about 2 hr. earlier than the maximum incorporation of calcium-45. The period of increased incorporation of calcium-45 is accompanied by the thermostabilization of structure of spores[3]. This observation is in accordance with the data from literature in which calcium bound with dipicolinic acid is assumed to be the main factor of thermoresistance of spores of bacilli[4–8].

The period of the formation of cysteine-rich structure precedes slightly the formation of the refractile structure of spores. A question arises whether the cystine structure contributes in some way to the resistance of spores. With regard to the possible importance of cystine in radioprotection, the resistance of sporulating cells of *Bacillus cereus* (NCIB 8122) to X-rays was examined. A liquid medium containing tryptic casein hydrolysate (0·3 per cent), glucose (0·1 per cent) and traces of some ions was used for cultivation, free spores having been used as inoculum. A vibration stirrer (100 oscillations/sec., amplitude 1 mm.) was used for splitting of chains, normally formed in this strain, and for homogeneous distribution of the air introduced into cultivation vessel. Practically only pairs of cells were present during the stationary phase of cultivation. Continually harvested samples of culture diluted with $M/40$ phosphate buffer to adequate concentration ($1 : 10^5$) were irradiated by Machlett *AEG*-50-*T* röntgen ray tube (50 kV., 40 m.amp., 0·1 mm. aluminium) in dishes (diam. 23 mm.) equipped with electromagnetic stirrers. On the basis of inactivation curves of vegetative cells and of free spores of this strain of *Bacillus cereus*, the radiation dose corresponding to 120 kr. was chosen. For detection of the loss of thermosensitivity of sporulating cells the aliquots were heated to 65° C. for 15 min. The number of surviving forms was determined in either case by plating procedure, non-treated suspension being used as control. As a recovery medium Difco nutrient agar was used. The percentage of spores was determined by direct microscopic observation and counting. The term 'refractile spores' is used for the more or less refractile areas of cytoplasm of the size and shape corresponding to spores, with somewhat diffuse rim. Cystine content was determined in acid hydrolysates of hot trichloracetic precipitates[3] by means of Shinohara's method[9] using phospho-18-tungstic acid.

The results are given in Fig. 1. They show that the radioresistance of sporulating cells appears simultaneously with the formation of cystine structures. Appearance of radioresistance is closely followed by the formation of refractile structure. The thermostabilization proceeds only after the fully refractile structure has been formed. With regard to the presence of pairs of cells in the culture, the results should be accepted as rather qualitative than quantitative. The correlation observed, however, between appearance of radioresistance and formation of cystine structure seems to be important.

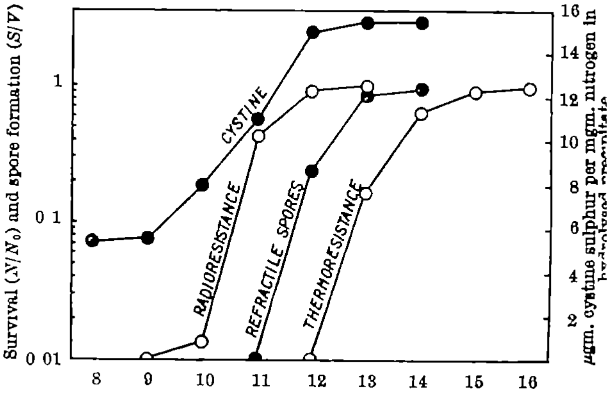

Fig. 1. Changes in radioresistance, cystine content and thermosensitivity of sporulating cells of *Bacillus cereus*. N, No. of surviving pairs of cells; N_0, No. of all pairs present; S, No. of cells with spores; V, No. of all cells present

Practically no cysteine has been found; only cystine is present in hot trichloracetic precipitates of spores of bacilli[3]. As the presence of disulphide linkages can be expected in proteins of spores, mention should be made of the role of this bond in radioprotection. According to results of paramagnetic resonance studies of X-irradiated proteins[10–12] the disulphide bond seems to have the function of a powerful electron donor. Whenever in proteins the electron is knocked out by ionizing radiation the vacancy created is assumed to be filled by an electron borrowed from a cystine group. The formation of mixed disulphides with protein SH—groups was demonstrated with typical radioprotective agents such as cystamine, cysteamine, etc.[13], and the radioprotective efficacy of these compounds is directly correlated to their ability to form disulphides[14]. It can, therefore, be expected that even in spores the disulphide groups play a prominent part in radioprotection of proteins. The cystine-rich protein structure, being localized in the integumental fraction of spores[3], can serve as a carrier for some important enzymes which were observed to be attached to the envelopes of spores[15,16].

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HISTOCHEMISTRY

Effects of Nitrogen Mustard on the Histochemistry of Mouse Lymphocytic Leukæmia *L* 1210

THE possibility of detecting chemical (histochemical) changes prior to discernible morphological changes in leukæmic cells following chemotherapy and the attempt to determine the emergence of cellular resistance to a given chemotherapeutic agent provide the basis of the present work.

Isotransplants of acute lymphatic leukæmia (*L* 1210)[1] and sub-lines resistant to 8-azaguanine developed in the Battelle Memorial Institute by the use of 8-azaguanine[2] in *DBA* and *DBF*1 hybrid mice were employed. A total of 80 animals were examined during the course of this work. An inoculation of 0·2 ml. of diluted peritoneal fluid made to approximately 100,000 cells was injected intraperitoneally into host mice; survival-time was between 8 and 9 days after inoculation. Peritoneal fluid was obtained on the 8th day and routine films and supravital preparations prepared. Immediately following this step the animals were injected with 0·05 mgm. nitrogen mustard in 0·1 ml. of distilled water (an *LD*50 for a 20-gm. mouse)[3]. At 15 min. and 1 hr. following injection samples of peritoneal fluid were withdrawn from each animal and films prepared Each animal served as its own control, and additional controls were used consisting of intraperitoneal injections of 0·1 ml. of distilled water.

The following histochemical methods were employed: (1) the DDD method for protein-bound sulphydryls[4]; (2) the indoxyl acetate method for non-specific esterase[5]; and the periodic acid–Schiff method for 1,2 glycols. Routine Wright's stained films and supravital preparations examined with the phase microscope were used for morphological evaluation.

The lymphocytic cells exhibited a strong reaction for sulphydryls localized in their cytoplasm, nucleoplasm and chromatin (Fig. 3). However, very occasionally morphologically intact lymphocytic cells were observed in the peritoneal fluid, which exhibited appreciably less reactivity for sulphydryls than the vast majority of leukæmic cells in the specimen (Fig. 4). In most instances, within 15 min. following the injection of nitrogen mustard there was a marked reduction in reactive sulphydryls present in all leukæmic cells (Fig. 5). This change in the sulphydryl reactivity occurred before the first morphological signs of cellular damage (Figs. 1 and 2), which usually appeared between 1 and 1½ hr. after the administration of the drug. The sulphydryl reactivity of the leukæmic cells present in one of the leukæmic sub-lines resistant to 8-azaguanine appeared less than that observed in the leukæmic cells in the non-resistant strains and other 8-azaguanine-resistant sub-lines. These cells too revealed a reduction in sulphydryl reactivity following nitrogen mustard, although the apparent degree of reduction was less than in the non-resistant strains. Esterase activity demonstrable in the lymphocytic cells with the indoxyl acetate method appeared to increase following the administration of nitrogen mustard. Increased activity as compared with controls was noted at both the 15-min. and 60-min. periods after therapy. Glycogen was not demonstrable in the leukæmic lymphocytes, although a few saliva-resistant periodic acid–Schiff-positive granules were present in their cytoplasm. Periodic acid–Schiff reactivity did not change following injection of nitrogen mustard.

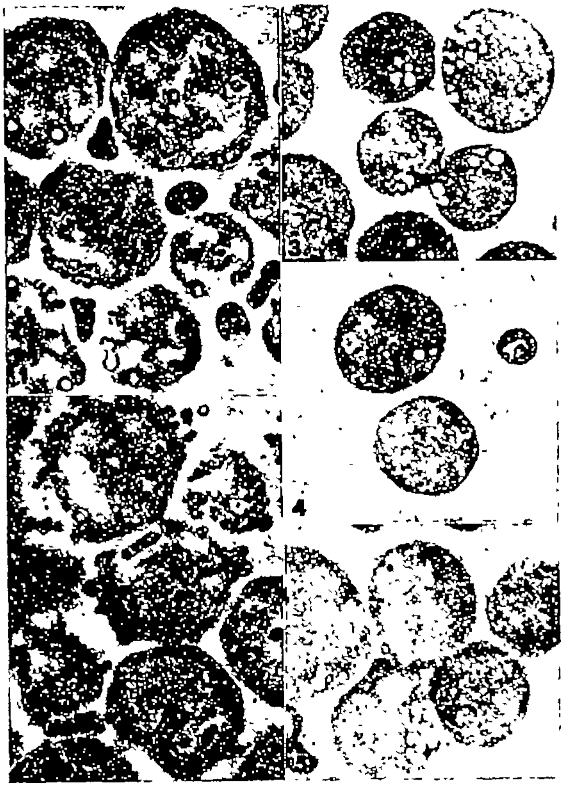

Fig. 1. Lymphocytic cells from peritoneal fluid. Non-resistant strain of mouse lymphocytic leukæmia *L* 1210. Control specimen. Phase contrast microscopy. Reduced from × 3,000

Fig. 2. Lymphocytic cells 1 hr. following intraperitoneal injection of nitrogen mustard. Note cytoplasmic budding. Phase contrast microscopy. Reduced from × 3,000

Fig. 3. Lymphocytic cells from non-resistant strain of mouse lymphocytic leukæmia *L* 1210. Control specimen. Note the strong sulphydryl reaction of these cells. DDD method for protein-bound sulphydryl. Reduced from × 3,000

Fig. 4. Similar to Fig. 3 except the lower cell exhibits a weaker sulphydryl coloration than the upper lymphocytic cells or those in Fig. 3. DDD method for protein-bound sulphydryl. Reduced from × 3,000

Fig. 5. Lymphocytic cells 15 min. following intraperitoneal injection of nitrogen mustard. Note the decrease in sulphydryl reactivity as compared with control specimen (Fig. 3). Reduced from × 3,000

This preliminary study indicates that chemical changes do occur and occur quite rapidly in mouse leukæmia cells of the *L*1210 strain following nitrogen mustard therapy. Certain histochemically demonstrable changes were noted in the leukæmic cells following nitrogen mustard and before apparent morphological alteration. The possibility of nitrogen mustard binding to available sulphydryl groups present in the leukæmic cells[6,7] seems likely, and may be the mechanism accounting for the decreased sulphydryl reaction given by these cells following nitrogen mustard. The possibility of histochemically distinct sub-lines of leukæmia and the possibility of histochemically detectable resistant cells in cell populations of non-resistant strains warrant further detailed study.

We wish to acknowledge the Battelle Memorial Institute for providing the animals employed in this investigation.

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Steroid-3β-ol-Dehydrogenase Activity in Fœtal Leydig's Cells

THERE is a considerable quantity of evidence demonstrating that the fœtal testis produces a hormone responsible, for example, for the freemartin effect[1]. Explantation of fœtal rat testes has shown that secretion of the hormone begins when the fœtus is 15–16 days old[2,3]. Morphological differentiation of the Leydig cells begins during the same period, the interstitial spaces being filled by closely packed polygonal cells containing periodic-acid–Schiff-positive material[4].

Although morphological observations thus favour the view that rat's Leydig cells are active and secreting from the third intra-uterine week, there is a lack of direct evidence for the presence in the testis of steroids or other substances leading to androgen formation.

In the work reported here a histochemical method[5] was used for the demonstration of steroid-3β-ol-dehydrogenase activity in the fœtal interstitial cells of the rat testis. This enzyme has been shown to play a basic part in the formation of the non-benzenoid steroid hormones[6]: consequently, its activity in the testis can be taken as an indicator of the hormone synthesis in it.

Female rats on heat were mated for 2–4 hr. Fœtuses were removed at intervals from 15 days after the mating, and either the whole fœtuses or their abdominal parts were rapidly frozen. Fresh sections were cut at 25μ with a cold microtome and stained with hæmatoxylin and eosin. Subsequent sections were incubated for the demonstration of the dehydrogenase activity. Dehydro*iso*androsterone in a concentration of 0·03 mgm./ml. served as the substrate and sections were incubated at 37° C. for ½–1 hr.

A positive dehydrogenase reaction was obtained in the rat fœtal testis from the fifteenth day of fœtal life. The interstices of the testes could then be identified with reasonable accuracy. In these, groups of cells giving a positive dehydrogenase reaction could be observed (Fig. 1). The sex cords did not show any enzyme activity at all. The formazan precipitate was finely granular in the round polygonal cells of the interstitial spaces, but a diffuse reddish diformazan staining of the interstices was also constant.

From the fifteenth day of pregnancy to a few hours after birth the histochemical reaction for steroid-3β-ol-dehydrogenase remained qualitatively similar, but quantitative changes could be noticed. The interstitial cells became more numerous and more closely packed, and, hence, the positive formazan deposition was more pronounced (Fig. 2). Formazan was still in the interstitial cells as a granular precipitate, and the germinal epithelium remained unstained. No certain increase in the intensity of staining of the individual Leydig cells towards birth could be observed. A positive dehydrogenase reaction occurred also in the Leydig cells of testes of rats killed a few hours after birth. There, the sex cords comprised a relatively larger portion of the testicle than during the fœtal life, and the positive interstitial cells were correspondingly more separated; but the dehydrogenase activity was otherwise unchanged in its localization and intensity.

The present observations give histochemical support to earlier findings of hormonal activity of the fœtal testes. It is of special interest to note that the steroid-3β-ol-dehydrogenase activity was found to continue from the fifteenth day to birth without any obvious changes in the enzyme activity in the individual Leydig cells, although the relative volume and number of these cells much increased during this period.

It has been proposed[4] that the different morphological appearance of pre- and post-natal Leydig cells would indicate different modes of secretion before and after birth. Our present findings show, however, that there is at least one important enzyme catalysing the synthesis of steroid hormones common in fœtal as well as in adult rat testicles. This does not, of course, prove that the fœtal testicular product was identical with the adult testicular hormone[7].

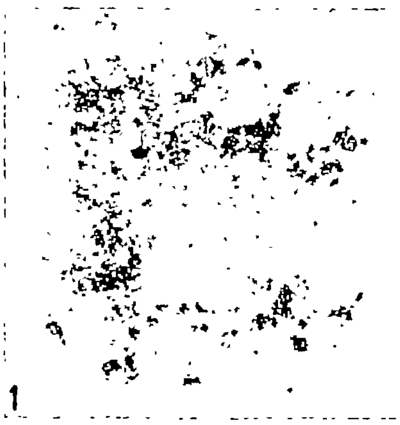

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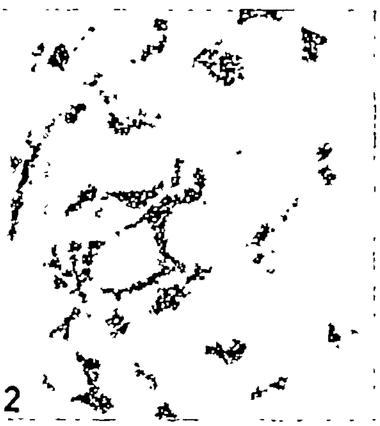
Fig. 1. Steroid-3β-ol-dehydrogenase activity in the testis of a rat fœtus, fifteenth day of fœtal age. Granular formazan deposits are visible in the interstitial spaces, the sex cords are unstained. (× *c.* 127)



2

Fig. 2. Testis of a fœtus of the eighteenth day. The Leydig cells are more numerous and further differentiated; the enzyme activity is clearly localized in the interstitial cells only. (× *c.* 77)

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PATHOLOGY

Mast Cells and Fibrinolysin

During the investigation of human tissues for fibrinolytic activity the presence of a fibrinolytic substance was detected in a specimen of a spleen. This was a case of splenic mastocytosis previously reported[1] in which there were many mast cells and a high concentration of histamine and heparin in the splenic tissue.

We investigated eighteen fresh samples of human spleens obtained at autopsy for the presence of plasminogen activator and plasmin-like activity using the selective and quantitative methods of Astrup, Mullertz[2] and Albrechtsen[3]. The fibrinolytic activity was recorded by measuring the diameter in sq. mm. of the products of tissue potassium thiocyanate extracts on standard bovine fibrin plates (heated and unheated). According to this method, the unheated bovine fibrin plates are reactive to both plasminogen activators and plasmin-like substances, whereas the heated plate reacts only to plasmin. It thus becomes possible to distinguish between plasminogen activators and plasmin or plasmin-like activity.

Potassium thiocyanate extracts of eighteen samples of eighteen different human spleens were assayed for fibrinolytic activity. Of the eighteen splenic extracts, only four showed some activity on heated and unheated plates but this activity was never equal to or greater than that activity of the mastocytotic spleen discussed in the above-mentioned case. Since the spleen containing mast cells had been kept frozen for more than a year when tested for fibrinolytic activity, it was felt that further work would be necessary to evaluate properly this fibrinolytic property.

It was decided to conduct additional studies on various tissues including mast cell tumours in dogs. All the dog tumours had portions removed for pathological examination. These were examined by hæmatoxylin and eosin stain and special stains for mast cells such as luna, toluidine blue and Bismarck brown.

All potassium thiocyanate extracts of dog mastocytomas reacted on unheated plates. On heated plates these extracts possessed little or no activity. One of the cases, a mast cell sarcoma, showed more tissue activator activity than any other tissue examined by us up to date. The tissue activator was found in varying amounts in dog tumours which contain little or no mast cells.

A different pattern of fibrinolytic activity emerged when carefully weighed samples of mast cell tumour tissue were placed on heated and unheated bovine fibrin plates[4,5]. All primary samples of mastocytoma tissue reacted on heated and unheated plates. It was interesting to note that the second and third recurrence of a mast cell sarcoma failed to lyse the heated fibrin plates. No other tumour, whether it was benign or malignant, lysed the heated fibrin plate. These observations are reported in Table 1.

Table 1. Fibrin Plates

Type of dog tumour	Unheated	Heated
Mast cell sarcoma		
Original	+++	+
First recurrence	+++	++
Second recurrence	+++	neg.
Third recurrence	+++	neg.
Malignant melanoma	++	neg.
Mast cell tumour	+++	+++
Mast cell tumour	+++	++
Myosarcoma	neg.	neg.
Breast fibro adenoma	++	neg.
Perianal gland adenoma	neg.	neg.

In accordance with interpretations of the above findings, it appears that these mast cell tumours contain a plasmin-like substance which dissolves both heated and unheated plates.

A study was made of the intracellular distribution of the fibrinolytic activity of the mast cell sarcoma noted in Table 1. Tissue activator was found in the low-speed sediment, the mitochrondrial and microsomal fractions. There was little or no activity associated with the soluble fraction. The microsomes contained the greatest concentration of tissue activator. A significant amount of plasmin-like activity was associated with the low-speed sediment.

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Pyridine Nucleotide Metabolism in Heliotrine Poisoning

It has been claimed[1] that failure of activity of a group of pyridine nucleotide - linked respiratory enzymes is an important factor in the acute hepatotoxic action of the alkaloid heliotrine, a member of the pyrrolizidine (*Senecio*) group. The respiratory effect occurred between 16 and 20 hr. after administration of the dose, and reactivation of the non-respiring mitochondria was obtained by addition of diphosphopyridine nucleotide (DPN) to the incubation medium.

Direct estimation of DPN and DPNH in liver homogenates of rats injected intraperitoneally with 320 mgm./kgm. body-weight of heliotrine was carried out by the alcohol dehydrogenase method[2], and a progressive fall to one-half of control values occurred over the period critical for respiration (Table 1). The assay method was not sufficiently sensitive for reliable determinations of mitochondrial DPN, although considerable reduction was observed over the same period.

Table 1. MEAN VALUES (μGM./GM. WET WEIGHT) OF LIVER HOMOGENATES OF RATS TREATED WITH HELIOTRINE AT VARIOUS TIMES PRIOR TO KILLING COMPARED WITH CONTROLS

Control	Heliotrine-treated		
	10 hr.	17 hr.	24 hr.
350	300	235	175

The possibility that heliotrine might reduce the capacity of the liver to synthesize pyridine nucleotides was therefore investigated. Initially, the DPN pyrophosphorylase activity of isolated nuclei[3,4] from control and heliotrine-treated rat liver was measured[5], and showed a definite but minor fall (Table 2).

Table 2. COMPARATIVE RATES OF DPN SYNTHESIS BY LIVER NUCLEI FROM CONTROL AND HELIOTRINE-TREATED RATS (μMOLE DPN/HR./10^{9} NUCLEI)

Animal	11 hr.	15 hr.	19 hr.
Control	23·1	22·7	21·7
Heliotrine-treated	19·4	17·5	13·1

Inasmuch as *in vitro* conditions, with optimum concentrations of substrate, might not reflect the full extent of an *in vivo* reduction of DPN synthesis, the degree of increase of liver DPN after injection of nicotinamide[6] was determined with control and heliotrine-poisoned animals. In the strain of Wistar rats used, an injection of 500 mgm. nicotinamide/kgm. body-wt. raised liver DPN several fold within 4 hr., to a limiting value which was maintained for many hours (Table 3).

Table 3. EFFECT OF NICOTINAMIDE (500 MGM./KGM. BODY-WEIGHT) ON LIVER DPN (μGM./GM. WET WEIGHT). EACH VALUE IS A MEAN OF DETERMINATIONS ON THREE DIFFERENT ANIMALS

Control	Nicotinamide-treated			
	4 hr.	8 hr.	12 hr.	18 hr.
285	810	1,015	1,020	915

With the animals pretreated with heliotrine, the ability to respond to a nicotinamide injection was depressed (Table 4), supporting the results with isolated nuclei (Table 2).

Table 4. MEAN LIVER DPN-LEVELS (μGM./GM. WET WEIGHT) 4 HR. AFTER NICOTINAMIDE INJECTION IN RATS PRETREATED WITH HELIOTRINE. TIMES REFER TO DURATION OF HELIOTRINE TREATMENT PRIOR TO KILLING ANIMAL

Control	Heliotrine-treated				
	4 hr.	14 hr.	18 hr.	20 hr.	24 hr.
810	750	715	580	545	375

In further experiments in which heliotrine-treated animals were given repeated injections of nicotinamide prior to killing, thus maintaining homogenate concentrations of DPN above normal values, the mitochondria failed to oxidize citrate, α-oxoglutarate and β-hydroxybutyrate unless DPN was added to the Warburg flasks. Chelating agents were ineffective.

Thus, although heliotrine produced a definite reduction of DPN synthesis at the 14–24 hr. period, this effect was not great enough to account for the complete failure of pyridine nucleotide-linked respiration observed at this time, and which still occurred even when homogenate DPN values were maintained above normal levels by administration of nicotinamide.

These observations support the hypothesis that failure of cell respiration in heliotrine poisoning is due to inability of the mitochondria to maintain effective local DPN-levels at enzymatically active foci, rather than to reduction of pyridine nucleotide synthesis.

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ANATOMY

Properties of Stressed Bone

DURING the past two years I have been investigating experimentally and histologically the relationship between stresses upon the skull, especially its sutures, caused by and transmitted through the masticatory apparatus.

The animals used in these experiments were the common South African vervet, *Cercopithecus aethiops*, and the stresses applied on the intact heads of the dead monkeys were by means of many ordinary elastic bands stretched around the skull and mandible.

Sections taken through various suture systems and the tooth-bearing parts of the jaws were fixed in 5 per cent formo-saline and decalcified in nitric acid. The sections were then treated with several of the conventional stains, including Masson's stain.

The pattern of green, reddish-brown and the mixture of these colours was so bizarre that it appeared at first to be completely random; but repeated instances of alterations in the colour from green to reddish-brown were found to be systematically recurring in the bones. They occurred according to whether or not stress had been applied to or transmitted through the bone, in that particular region.

When Masson's stain combination is applied to a section of unstressed bone the calcified tissue stains green. If the section passes through an area stressed in the manner stated above the colour of the stressed region is found to be reddish-brown.

The regularity of this localized colour change was noted and then Nicol's prism demonstrated that the normally green staining bone does not transmit light, but that the stressed reddish-brown staining bone now transmits light. Clearly a fundamental physical change had accompanied this profound alteration in staining reaction.

Lest the stain itself should be responsible for the physical change, serial sections of the same specimen were examined for birefringence. Both unstained sections and those stained with hæmatoxylin and eosin (which display no such colour differentiation as exhibited by the Masson-stained specimens) are found still to demonstrate the birefringence caused by the preceding stress.

Sections of the neurocranial bones, which have not been stressed experimentally prior to preparation and Masson's staining, sometimes show an outer plate to which muscles are attached and which exhibit a reddish-brown staining accompanied by birefringence while the inner table is almost entirely green. Where the inner table happens to be reddish-brown it too is birefringent.

Similar changes in the birefringence and staining properties of stressed bone have also been demonstrated in the long bones of vervet monkeys. This indicates that the changes brought about *in vitro* probably also occur *in vivo*. If so, these physical changes in bone must be reversible and perpetually recurring according to the stresses exerted by external forces such as those applied experimentally here or by forces such as those which are developed during muscular activity.

This communication has been prepared to direct attention to some of the changes that occur in bone as a result of being stressed. These changes may have an important bearing on the growth and repair of bone.

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BIOLOGY

Transfer of Tolerance to Skin Grafts in F_1 *C57B*1 Inbred Mice

Co-existence of adult animals in experimental parabiosis is followed in due time by 'immunological neutralization' as revealed in tolerance to reciprocal skin grafts. Nakic and Silobrcic[1] observed in rats of different breeds that even after 5 days of parabiotic union homografts survived a longer time than in control animals. Skowron-Cendrzak and Konieczna-Marczyńska[2] reported that the skin homografts of white, non-related mice 'took' permanently after seven weeks of parabiosis. Rubin[3] and Martinez *et al.*[4] found parabiotical neutralization in hybrids of two inbred mice-strains united with individuals of one of the parental breeds. The survival-time of grafts of separated mice parabionts depends upon the length of time of parabiotic union. Similarly, it has been possible to obtain with the help of parabiosis permanent skin isografts in mice characterized by the strong sex-antigen which normally counteracts successful male skin transplantation to the female[5,6]. The duration of parabiosis necessary to evoke neutralization depends upon the degree of antigenic differences between the united individuals. It was shown that in *C57B*1 inbred mice which only differ in sex-antigen, skin grafts from the male to the female performed one week before union may already 'take' permanently[7].

The mechanism of neutralization has not yet been fully elucidated and can be conditioned by such different factors as trauma, enhancement, immunoparalysis and immunological unresponsiveness in adults. The induction of tolerance takes place only if the vascular connexion between the joined animals is established, and a constant reciprocal exchange of a great quantity of antigens exists. In long-term parabiosis between the individuals of two inbred strains of mice (*CBA*,*A*) in which neutralization was lacking, there was never any vascular connexion between parabionts (unpublished work).

The purpose of the work reported here was to establish whether the tolerance to male skin isografts acquired in females during parabiosis can be transmitted to the F_1 generation.

Mice of the inbred *C57B*1 strain imported by our Department three years ago from the Institute of Animal Genetics, Edinburgh, served as material. Parabioses were carried out, always uniting females with isologous males (sisters with brothers). The animals were 4–6 weeks old. One week after the establishment of parabioses, skin grafts were exchanged between the respective members of each parabiotic pair. Whole-skin grafts taken from the back measuring 2 cm. × 2 cm. and rotated 180° were kept in place by sutures. All skin isotransplants were successful. After 1–10 weeks the parabionts were separated and kept in pairs in order to obtain the F_1 generation.

Out of thirty-four F_1 females on which, at the age of 4 weeks, the male brother skin was transplanted, in two cases the grafts were accepted permanently. These grafts were held until the death of the animals (133 and 140 days respectively). In all other females (32) the great majority of isografts survived significantly longer than in controls ($t = 4 \cdot 51$; $P < 0 \cdot 01$). The end of the survival-time was taken to be when the first signs of incompatibility reaction appeared. The control group consisted of fifty *C57B*1 females, 4–6 weeks old, on which the skin taken from isologous male donors of the same age was transplanted. In fourteen females, 2–3 weeks after the first graft, the second-set grafts from the same males were performed. A much shorter survival-time of the second-set grafts corroborates the results obtained by Eichwald *et al.*[8] and reveals the immunological nature of the graft rejection.

The results of isotransplantation in control as well as in experimental mice are shown in Table 1.

Table 1. Survival-Time (days) of ♂ to ♀ Skin Isografts in Strain *C57B*1 Mice

Group		No. of animals	Range	Mean	Standard deviation
Control	First graft	50	12–18	15	± 1 5
	Second graft	14	6–11	7 6	± 1 3
Experimental	First	32	14–49	21	± 9·1

In transmission of tolerance to the F_1 generation the duration of union of parental individuals plays no part. This is evident from the fact that permanent acceptance of the male skin grafts was observed in one of the F_1 females of which the parents had only been united during one week. Similarly, the length of time after separation of parabionts probably has no influence on the transmission of tolerance. The second female, permanently tolerant to the male skin graft, belonged to the animals of the F_1 generation, which were born not earlier than 8 months after the separation of the parabionts. From one and the same parental pair and even from only one individual of them, females can be born which are either completely tolerant, incompletely tolerant or which reject male skin within the same period of time as the control animals do.

At present further experiments are being carried out on isografts of the F_1 and F_2 generation in order to elucidate both the mechanism of parabiotic neutralization and the mechanism of tolerance transmission.

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Perfect Stages of *Fusarium oxysporum* and of *Fusarium solani* f. *pisi* still Unknown

A YEAR ago Buxton[1] reported the development of red, *Nectria* perithecia after ultra-violet irradiation of a culture of *Fusarium oxysporum* f. *pisi* (Linf.) Snyd. and Hans. He stated that the fungus causes typical wilt of pea, was re-isolated from the reddened vascular tracts of wilted plants and that, therefore, there could be little doubt but that this homothallic *Nectria* is the perfect stage of that particular wilt-inducing *Fusarium* isolate (that is, *F. oxysporum* f. *pisi*).

Recently, Gordon[2] has taken issue with Buxton's claim, pointing out quite clearly and correctly that *Nectria* (=*Hypomyces*) is well known as a perfect stage of some clones of *F. solani* (Mart.) App. and Wr. emend. Snyd. and Hans., but that for various reasons it would not be expected as a perfect stage of *F. oxysporum*.

We have examined a culture of Buxton's fungus, containing the *Nectria* perfect stage, which had been deposited with the Commonwealth Mycological Institute at Kew. We find that this fungus is a typical, homothallic *Hypomyces solani* Rke. and Berth. emend. Snyd. and Hans. (=*Nectria haematococca* Berk. and Br.), as diagnosed by the Commonwealth Mycological Institute, and Gordon, and has as its imperfect stage *Fusarium solani*. Repeated single-spore cultures made by us of both the ascospores and conidia from the Buxton culture yielded the same, homothallic species. Perithecia developed abundantly within two weeks after transfer and required only diffuse daylight for their initiation and maturation. In these respects we are in agreement with Gordon, and likewise we have not found *F. oxysporum* in the culture, just *F. solani*. These two species are very distinctive and readily differentiated in culture. Over a period of decades during which these two fungus species have been studied, no conclusive evidence has been presented, either of overlapping of the species or of their common *Hypomyces* origin.

Gordon[2] has expressed the opinion that Buxton did not have *F. oxysporum* f. *pisi* but rather *F. solani* f. *pisi* (Jones) Snyd. and Hans., and postulated that the perfect stage in *Hypomyces* (=*Nectria*) which Buxton reported is that of *F. solani* f. *pisi*. Further, that since the perfect stage of this pathogen is unknown, Buxton's report represents the first record of its occurrence.

We do not agree with Gordon's postulation. We have run two separate inoculation tests in the greenhouse on two varieties of pea. In these tests three fungi were used: Buxton's isolate, a similar homothallic *Hypomyces solani* indirectly obtained from the Rothamsted area, England, some ten years ago, and a local isolate of *F. solani* f. *pisi*. Inoculations were made by applying spore suspensions to pea seedlings growing in pots of sterilized soil. In both tests, run at different times, the plants inoculated with Buxton's fungus, the Rothamsted fungus, and the uninoculated controls were completely healthy. Neither cortical root and stem rot nor vascular wilt symptoms were present. Those plants inoculated with *F. solani* f. *pisi*, however, all showed typical brown to blackish lesions completely covering the two inches of underground hypocotyl of each plant.

Our conclusion is that the *Hypomyces* (=*Nectria*) reported by Buxton to be the perfect stage of *F. oxysporum* f. *pisi* is not the perfect stage of this fungus, nor the perfect stage of *F. solani* f. *pisi*. We suggest that this isolate is a homothallic, saprophytic fungus with an imperfect stage in *F. solani*. In our opinion, therefore, no perfect stage is yet known for any clone of *F. oxysporum*, nor for any clone of the pea root and stem rot form, *F. solani* f. *pisi*.

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Effect of 6-Methoxybenzoxazolinone on the Growth of *Xanthomonas stewartii* (Erw. Smith) Dowson and its Presence in Sweet Corn (*Zea mays* var. *saccharata* Bailey)

PREVIOUSLY[1] it was reported that 6-methoxybenzoxazolinone (MBOA) inhibited growth of fungi associated with root and stalk rot of field corn. This communication reports the inhibitory effect of MBOA on *Xanthomonas stewartii*, the causal organism of bacterial wilt (Stewart's disease), and the presence of this compound in the sweet corn plant.

The effect of various concentrations of MBOA in agar (beef extract 3 gm., agar 17 gm., distilled water 1,000 ml., adjusted to *p*H 6·0) on the growth of *X. stewartii* after 4 and 8 days is shown in Table 1. Inhibition of growth occurred at 0·15 mgm. per ml.,

Table 1. GROWTH OF *Xanthomonas stewartii* ON AGAR (CHECK) AND ON AGAR CONTAINING MBOA

Concentration of MBOA (per ml. of agar) (mgm.)	Growth Days after inoculation 4	8
0 (check)	+++	+++
0·075	+++	+++
0·15	+	++
0·3	—	+
0·6	—	—

+++, maximum growth; —, no growth.

and the material was bactericidal at 0·6 mgm. per ml.

An ether extract of juice from the roots and stalks of two sweet-corn hybrids, prepared by the method reported earlier[2], significantly inhibited growth of *X. stewartii*. A chemical spot test for the determination of MBOA, in which diatized sulphanilic acid and sodium carbonate mixed with MBOA gives a characteristic reddish spot on filter paper, showed this compound to be present in the extract.

The fact that MBOA inhibits growth of *X. stewartii* and is present in the ether extract of sweet-corn plants suggests that this compound is a possible factor in resistance to bacterial wilt.

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Location of Manganese taken up in Short-term Absorption by Oat Roots

The oats (variety Sun II) used in experiments reported here were grown on waxed nylon mesh and supplied with Hoagland and Arnon's[1] culture solution modified by the omission of manganese chloride from the micronutrients. The roots were cut off below the netting when about 3–5 cm. long. They were washed with distilled water and immersed in a large volume of $M/1,000$ manganese sulphate solution for 2 hr.

At the end of this time the excised roots were washed with distilled water and stained by a technique based on that of Batalin[2]. The roots were treated as follows: 10 per cent aqueous potassium hydroxide solution for 5 min., removal of excess liquid by blotting, irrigation with 5 per cent *p*-anisidine solution for 5 min., and finally acidification with dilute sulphuric acid to reveal the presence of manganese by a purple coloration.

This procedure showed that most of the manganese was concentrated in the meristematic zone immediately behind the root cap (dark area in Fig. 1). As the dye is water-soluble such preparations are only temporary.

To exclude the possibility of an artefact caused by differential uptake of the dye, roots similarly prepared were analysed for manganese by the method of Cornfield and Pollard[3]. After absorption of manganese, and washing, the tips (about 2–3 mm.) were cut off, and the two portions sucked dry on a sintered glass filter, weighed and analysed. Manganese contents (as parts per million of fresh weight) are shown in Table 1.

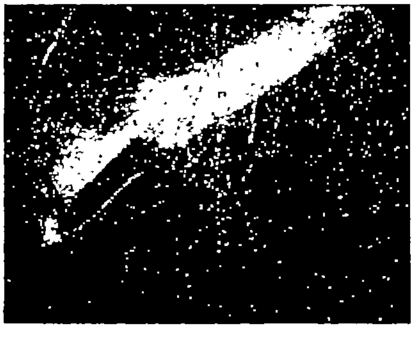

Fig. 1

Table 1

	Manganese absorbed by· Complete excised roots	 Tips and remainder separately
Tips (2–3 mm).	400 p p.m.	260 p.p.m.
Remainder of roots	100 p p m.	130 p p.m.

To test the possibility of translocation within the root to the meristematic region after more dispersed uptake the experiment was repeated, but root tips and the remainder of the roots were separated before being placed in two portions of $M/1,000$ manganese sulphate solution.

These figures are only approximate, since it is not possible, with the technique described, to separate efficiently the meristematic zone from the rest of the root.

Nevertheless the results show sufficiently clearly that: (*a*) short-term uptake of manganese occurs more rapidly in the meristematic region of the oat root; (*b*) translocation of manganese occurs within the root, since when the tips are separated, their uptake is lower, and that of the rest of the root higher, than when uptake occurs in the complete excised root.

It is a pleasure to record thanks to Prof. W. W. Fletcher and other members of the Botany Department of this College for providing microscopic and photographic facilities and other help, and to Dr. Ben-Zion Ginsberg (The Hebrew University, Jerusalem, now on a visit to the University of Edinburgh) for helpful discussion.

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Relationship of Radiation, Leaf Area Index and Crop Growth-Rate

Shading experiments, such as those by Blackman and Templeman[1] on white clover, have shown the great reduction in crop growth-rate which occurs when light intensity is curtailed. A second factor influencing crop growth-rate is the leaf area index. There is evidence[2,3] that the rate of dry matter production by a crop or pasture will increase as the leaf area index increases, until a maximum value is attained; thereafter as the index increases further, the rate of dry matter production will decline. This is presumed to occur because the lowermost leaves become so heavily shaded at high leaf area index that their photosynthetic contribution is less than their respiration.

It is likely that these two inter-relationships, light and crop growth-rate, and leaf area index and crop growth-rate, may interact, and various workers, especially Monsi *et al.*[4], have presented theoretical views on this subject.

In the course of competition studies at Adelaide, results have been secured on this inter-relationship between the level of radiation, the leaf area index and the rate of dry matter production. An experiment was conducted to study the influence of shading by grass on the growth of an associate clover when the roots of the two species were kept separate and there was competition only for light. Each species was grown in narrow, slot-like soil compartments; clover swards comprised a number of 'clover slots' placed together, while mixed swards were made up of alternating 'grass slots' and 'clover slots'. The percentage daylight at the surface of the clover canopy was determined by the use of a small selenium cell on a probe[5] and this percentage was applied to the values for total radiation recorded by a nearby Kipp solarimeter. In pure cultures of clover the surface light intensity was the full daylight; in the mixed cultures of grass and clover the light intensity at the surface of the clover was derived from the profile of the light intensity (based on readings at 2 cm. vertical intervals) and a measurement of the mean height of the upper clover leaves. Frequent harvests permitted calculation of the growth-rate of the clover during successive 14-day intervals. The experiments were done in the winter period June–August 1959; the weekly mean temperatures during these months showed unusually low variation (54·3° F. ± 2·7° F.).

The results of this work indicate that for any level of radiation there is an optimum leaf area index, at which crop growth-rate will be maximal, and that the value of the optimum index increases as radiation rises. These concepts are presented in Fig. 1 in which isopleths for crop growth-rate are drawn for varying levels of radiation and leaf area index. With a radiation of 50 gm.cal./cm.^{2}/day the optimum leaf area index would be about 3·5, and the crop growth-rate low (0·07 gm./dm.^{2}/day), whereas with a radiation of 150 gm.cal./cm.^{2}/day the optimum leaf area index would be about 5, and the crop growth-rate relatively high (>0·1 gm./dm.^{2}/day). The broken line indicates the rates of production when varying levels of radiation are associated with optimum values of leaf area index. To the left of this broken line, light interception is incomplete; to the right of the line, some of the lower leaves are below compensation point.

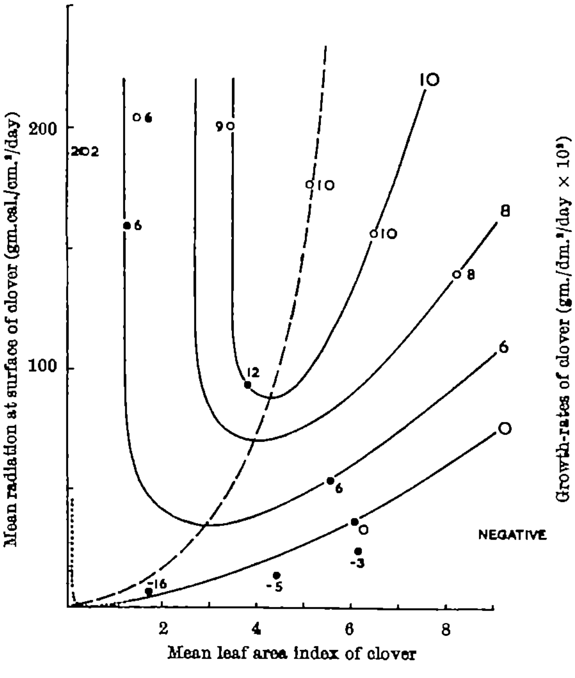

Fig. 1. ○, In pure stands; ●, in mixed stands

When shading of the clover by grass was severe, the entire clover community was below compensation point and negative values for growth-rate were recorded. The zero isopleth for crop growth-rate shows the level of radiation needed to keep a clover sward of any particular leaf area index at compensation point. It is suggested that extrapolation of this line to the lowest index values would follow the course shown by the dots—that is, it would turn sharply at the minimum index required to maintain plants alive. The part of the graph in which the isopleths are vertical and parallel is the zone in which an increase in radiation would give no further increase in growth-rate of a sward of any particular leaf area index.

The data are not sufficient to establish these relationships with precision but it suggested that they indicate the nature of the interactions in pasture swards and presumably also in crops and other plant communities.

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Possibility of Fixation of Atmospheric Nitrogen by the Rice Plant

A RECENT communication[1] by Chakraborty and Sen Gupta suggested that rice plants might be capable of fixing atmospheric nitrogen. We have made an attempt to confirm their result using the variety 'Lead 35' (a pure-line selection of material derived from British Guiana). No evidence of nitrogen-fixation has been obtained.

Chakraborty and Sen Gupta found that the maximum rate of nitrogen-fixation occurred in young plants—a gain of 50 per cent of the nitrogen content of 7-week-old plants being recorded after a further month in nitrogen-free medium. The percentage gain in older plants was substantially lower. For this reason our experiments have been carried out on young plants.

Rice plants were grown from seed in trays. Each tray of 30 seedlings contained 2 l. of nutrient medium of the following composition, changed at fortnightly intervals:

$MgSO_4.7H_2O$	0·35 gm./l.
$Ca(NO_3)_2$	0·92 ,,
KH_2PO_4	0·38 ,,
KNO_3	0·85 ,,
Ferric ethylenediamine tetraacetic acid	0·07 ,,
H_3BO_3	1·86 mgm./l.
$ZnSO_4.7H_2O$	0·29 ,,
$MnSO_4.4H_2O$	2·23 ,,
$(NH_4)_6Mo_7O_{24}$	0·035 ,,
*p*H	about 4·5

The whole apparatus was enclosed in a polythene cloche to prevent contamination of plants or medium by rain-water, dust and insects. Plants were grown for an initial period in complete medium, then removed and their fresh weights determined. After washing in three changes of distilled water, one batch was analysed by the micro-Kjeldahl method and the remainder were returned to the dishes to be grown for a further period, this time in a nitrogen-deficient medium similar to that used in the first period but with the omission or substitution of nitrogenous constituents. At the end of the second period, these plants were removed and analysed together with their dead leaves, which had been collected and preserved during the period of nitrogen starvation. The results were assessed by plotting fresh weight at the beginning of the period of nitrogen starvation against the Kjeldahl-nitrogen content before and after nitrogen starvation. Regression lines were then calculated for nitrogen content on fresh weight for each set of plants.

In the first experiment, 68 plants were grown, 44 analysed after the first three weeks and 24 after a further three-week period of nitrogen starvation. The equation for the regression line after the initial period was $y = 5{\cdot}49x - 0{\cdot}11$ ($r = 0{\cdot}976$), and after the period of nitrogen starvation $y = 6{\cdot}19x - 0{\cdot}52$ ($r = 0{\cdot}914$). Statistical comparison of these lines showed that there was no significant difference between their slopes and that no significant increase in the nitrogen content of the second batch had occurred. This was to be expected as marked deficiency symptoms had become apparent in the plants after they had been in the nitrogen-deficient medium for a week: leaves became chlorotic and eventually died from the tips. However, as heavier plants in this experiment had shown a tendency towards apparent gain in nitrogen content, a second experiment was carried out in which the initial period was increased to 4½ weeks. 52 plants were grown, of which 29 were analysed after the first period and 23 after nitrogen starvation. The results are shown in Fig. 1. The nitrogen contents of the starved plants were significantly higher than would be predicted from the first regression line. The apparent gain amounted to 17·7 per cent of the average initial nitrogen content per unit weight of fresh plant material. As the plants had again shown severe deficiency symptoms after a period of one week of nitrogen starvation, it was considered that an alternative explanation to that of nitrogen-fixation was likely. An attempt was made to determine the nitrate content of similar plants to discover whether nitrate reserves—not measurable by the ordinary micro-Kjeldahl method—were being used up during the period of nitrogen starvation and rendered amenable to determination by the Kjeldahl method, resulting in an increase in the measured nitrogen content of the plant.

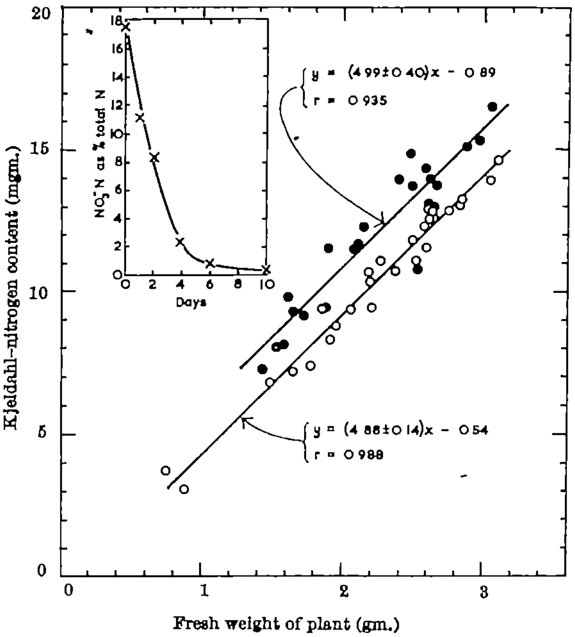

Fig. 1. Relationship between fresh weight of plants 4½ weeks old at the beginning of the period of nitrogen starvation and the Kjeldahl-nitrogen content before (○) and after (●) 3 weeks of nitrogen starvation. Inset: Depletion of nitrate in 4-weeks-old plants transferred to nitrogen-deficient medium

Rice plants were grown as before for an initial period of 4 weeks. Analysis of 9 plants by the original micro-Kjeldahl method gave nitrogen contents comparable with previous results. Six plants from the same batch were dried at 105° C., extracted for 1 hr. with boiling water, and the extract distilled using magnesium oxide and Devarda's alloy for the determination of ammonium nitrogen. The content of nitrate nitrogen was found to be 17·5 per cent of the total nitrogen content of the plant. This figure is in agreement with the apparent gain in the previous experiment. We have found that the nitrate content of older plants is less: for example, 7-week-old plants contained 10·7 per cent of their nitrogen as nitrate. Reliance on ordinary Kjeldahl-nitrogen determinations would therefore result in an apparent decrease in 'fixation' in older plants. But we have also found that the nitrate content of plants grown in hydroponics is abnormally high when compared with rice plants growing under natural conditions: using the same analytical method, measurable quantities of nitrate nitrogen could not be detected in rice plants growing under field conditions, even when plants were sampled at daybreak from plots which had received heavy dressings of nitrogenous fertilizer.

Fifteen of the 4-week-old plants were also transferred to the nitrogen-deficient medium from which they were afterwards removed at intervals and their nitrate content determined. The results are shown in the inset to Fig. 1. Severe symptoms of nitrogen deficiency were apparent after 7 days. At this stage the nitrate reserves of the plants were almost exhausted.

The results demonstrate that caution is necessary when applying the ordinary Kjeldahl methods to plants grown in hydroponics because of the unexpectedly high nitrate contents which may be found in such plants.

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ENTOMOLOGY

Enzymic Glucoside Synthesis in Locusts

INSECTS are known to convert foreign phenols to glucosides[1], and a few normally occurring glucosides have been identified in insects[2-4]. In other organisms the sugar for analogous glycoside synthesis comes from uridine diphosphate sugar derivatives and a similar process appears to function in insects the uridine diphosphate glucose (UDPG) of which provides the glucose for trehalose[5] and arbutin[6] formation. We have now investigated the distribution and extent of this mechanism and the specificity of its need for UDPG in the locust, *Schistocerca gregaria*.

The aglycone used as substrate was 4-methylumbelliferone, which forms fluorescent glycosides which may be hydrolysed by snail gastric enzyme or acid to the highly fluorescent phenol. Samples of reaction mixtures were chromatographed on No. 4 Whatman paper in 2-butanone/2 *N* ammonia (1 : 1, v/v) to separate the glucoside (R_F 0·6) from unchanged aglycone (R_F 0·8). The glucoside zone, located by examination under ultra-violet light, was eluted with *N* hydrochloric acid and estimated by measurement of its fluorescence at 410 mμ when activated at 320 mμ in an Aminco-Bowman spectrophotofluorimeter.

When separate tissues of fifth instar hoppers or adult locusts were examined, little conjugating activity was found in organs other than the fat body (Table 1). Measurements were also made of the conjugating capacity of whole locust carcasses after removal of the gut and Malpighian tubes. Locusts obtained on different occasions varied widely in their capacity but rates of formation of glucoside up to 250 μgm./hr./locust were found. For these experiments the carcasses were suspended in 5 ml. of saline[7] containing 0·5 mgm./ml. of methylumbelliferone and 0·1 ml. aliquots removed periodically for analysis. The concentration of glucoside increased linearly with time for at least 2 hr. and no difference in rate was observed with air or oxygen in the gaseous phase. The saline solution used contained 0·8 per cent (w/v) of glucose but omission of the sugar or replacement with equimolar amounts of trehalose or sucrose made no significant difference in the rate of glucoside synthesis.

Table 1. DISTRIBUTION OF GLUCOSIDE-FORMING ACTIVITY IN LOCUSTS

Organ	Methylumbelliferone conjugated with glucose (μgm./hr./locust)
Washed foreguts	0·05
Washed gastric cecæ	0·06
Washed mid- and hind-guts	0·03
Malpighian tubules	0
Fat bodies	2·0
Flight muscles	0
Hæmolymph	0

Each tube contained 0·1 mgm. 4-methylumbelliferone dissolved in 0·2 ml. grasshopper saline (ref. 7) at *p*H 7 and the tissues from 5th instar hoppers. Tubes were incubated at 37° for 1 hr. in air.

When fat bodies were homogenized for 5–10 sec. in a glass Potter homogenizer, the conjugating activity was reduced to very low levels but this could be restored to normal by addition of UDPG to the medium (Table 2).

Table 2. EFFECT OF HOMOGENIZATION AND UDPG ON METHYL UMBELLIFERONE GLUCOSIDE SYNTHESIS IN LOCUST FAT BODY

Experiment	Rate of glucoside formation (μgm./hr./locust)
1. Fat body suspension (3 fat bodies/ml.)	10·4
2. Fat body suspension after homogenizing	0·9
3. Fat body suspension after homogenizing and addition of UDPG 1 mgm./ml.	12·9

Fat bodies were suspended by shaking in saline and 1 ml. suspension used for experiment 1. The rest of the suspension was homogenized and 1 ml. of homogenate used for experiments 2 and 3. 4-Methylumbelliferone was added to each tube to give a concentration of 0·5 mgm./ml.

Homogenized fat bodies were centrifuged at 500–1,000 r.p.m. and the aqueous layer used as enzyme in determination of optimum substrate concentration and *p*H. The reaction showed a broad *p*H maximum at 7–8 and at this *p*H and with a methylumbelliferone concentration of 0·5 μgm./ml. the maximum rate of reaction was found with 1 mgm./ml. of UDPG.

Uridine diphosphate N-acetylglucosamine, which may be involved in chitin formation, was substituted for UDPG in similar experiments but no evidence was found of any glucosaminide formation. Similarly, UDPGA (uridine diphosphate glucuronic acid), which is used in analogous vertebrate detoxication reactions, gave no glucuronide when incubated with methylumbelliferone and intact or homogenized locust carcasses or fat bodies. In experiments where 0·1–1·0 equivalents of UDPGA were added to reaction mixtures containing the optimum concentration of UDPG, no inhibition of glucoside formation was observed.

This work was supported by grants from the Anti-Locust Research Centre.

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Fate of Aldrin and Dieldrin in Locusts

ALDRIN and dieldrin are widely used as insecticides, and dieldrin has been employed against locust hopper bands in the field. Aldrin is known to be epoxidized to dieldrin in insects[1,2], in vertebrates[3], in soils[4] and on plants[5]; but in many cases analytical difficulties have left the extent of the change not clear. The availability of aldrin and dieldrin labelled with chlorine-36 has now allowed us to examine quantitatively the metabolism and excretion of aldrin and dieldrin in *Schistocerca gregaria*.

Fifth instar hoppers were injected abdominally with an olive oil solution of the insecticides (2 μgm./5 μl.; about 100 c.p.m.) and kept at room temperature. Any insects surviving after seven days were added to the dead, which had been preserved under

Table 1. EXCRETION OF ALDRIN AND DIELDRIN BY LOCUSTS

Material used	Percentage of dose excreted	Percentage of chlorine-36 in bodies as: aldrin	dieldrin	water-soluble material	Percentage of chlorine-36 in excreta as: aldrin	dieldrin	water-soluble material*
Aldrin 1	3·3	62	39	0	44	52	5
2	21	67	36	0	57	41	3
3	—	—	42	—	—	—	—
Dieldrin 1	22	0	101	0	—	—	—
2	—	—	—	—	—	99	0
3	21	0	101	0	—	108	0

2 μgm. was injected in olive oil solution and surviving insects killed after 7 days.
* Not chloride.

ethanol. Excreta from each batch of locusts were collected at the end of seven days and similarly preserved under ethanol. Dosing of batches of locusts was repeated weekly until a total of 40,000 c.p.m. had been injected.

Excreta or bodies were homogenized in the Atomix blender with ethanol and filtered. The residue was then extracted in turn with acetone, 2 *N* ammonia and 0·1 *N* hydrochloric acid and the pooled filtrates concentrated *in vacuo* and counted as 'infinitely thin' layers on aluminium planchettes with a thin end-window counter. The extraction procedure was repeated until all the injected activity had been recovered.

The pasty residue left on concentration of the extracts was partitioned between water and benzene, and any water-soluble material removed and counted after concentration. Aliquots of the benzene solution were taken for dilution analysis with aldrin or dieldrin.

Aldrin was re-isolated from the mixture and crystallized to constant specific activity. Its radioactive purity was checked by conversion to the 2,4-dinitrobenzene sulphenyl derivative, m.p. 264°, with the same equivalent specific activity. Dieldrin was re-isolated, and its radioactive purity confirmed by conversion to the antimony chloride complex, which had the same equivalent specific activity.

This derivative was prepared by mixing equivalent amounts of dieldrin and antimony trichloride in hot *n*-hexane solution, from which the complex crystallized on cooling. It formed colourless needles from hexane, m.p. 122°, but decomposed in polar solvents. Found: Sb 19·6, Cl^- 17·5, $C_{12}H_8Cl_6O \cdot SbCl_3$; required Sb 20·0, Cl^- 17·5 per cent. Antimony tribromide formed a similar complex, m.p. 131°. Found: Sb 16·4, Br^- 32·7, $C_{12}H_8Cl_6O \cdot SbBr_3$; required Sb 16·4, Br^- 32·3. Endrin also formed similar complexes but aldrin and isodrin gave no derivative under these conditions.

Aldrin and dieldrin were only slowly excreted by locusts (Table 1) and the rate of oxidation of aldrin (Table 2) was considerably lower than has been reported for flies (about 0·1 μgm./hr./fly) under similar conditions[1]. A small amount of water-soluble material was found in excreta but could not be detected in the bodies in spite of the much larger amounts of radioactive material there. This was shown not to be chloride by dilution analysis and is being further investigated.

Dieldrin itself was not further metabolized and was slowly excreted unchanged.

Repeated sub-lethal doses of dieldrin, in contrast to di-nitro-*o*-cresol[6], should therefore act cumulatively in locusts as has been found with flies[7].

We are grateful to Prof. R. T. Williams for a gift of the radioactive insecticides used in this work.

Table 2. OXIDATION OF ALDRIN TO DIELDRIN IN LOCUSTS

No. of locusts	Dose (μgm./locust)	Time after dosing (hr.)	Dieldrin formed (percentage of dose)	Rate of oxidation (μgm./dieldrin/locust/hr.)
12	1·8	44	22·7	0·011
12	1·8	60	89·1	0·012
12	1·8	72	42·0	0·011
29	1·8	(pooled dead)	42·2	—
7	0·9	98	54·0	0·005

5th instar locusts were injected with aldrin in olive oil and groups of 12 analysed for dieldrin at intervals. Insects dying at any time were pooled and analysed for dieldrin. No food was given and excreta were negligible.

The work was supported by grants from the Anti-Locust Research Centre.

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BACTERIOLOGY

Lipolytic Activity of Some Anaerobic Bacteria

THE use of the water-soluble 'Tweens' for easy detection of lipolytic activity by micro-organisms has already been described by me[1]. This technique has now been adapted to detect lipolytic activity of anaerobic bacteria. The best results were obtained by using the medium '*VF*' of Prévot with the addition of 'Tween 20', '40', '60', or '80' (polyoxyethylene sorbitan mono-laurate, mono-palmitate, mono-stearate and mono-oleate respectively) in a final concentration of 7 × 10^{-3} *M* plus 0·01 per cent (w/v) calcium chloride. After incubation at 37° C. the 'Tween' cultures were examined for the presence of opaque haloes, due to the formation of conspicuous precipitates of calcium laurate, palmitate, stearate or oleate around the zones of growth. The test was made either in tubes (8 mm. × 180 mm.) following the classical techniques for growing anaerobic bacteria,

Table 1. COMPARISON OF THE LIPOLYTIC ACTIVITY OF SOME ANAEROBIC BACTERIA AS SHOWN BY THE EIJKMAN AND SIERRA METHODS

The figures in brackets indicate the number of days after which a positive result could be observed. Cultures containing no lipolytic bacteria were observed during a month

Culture tested	Origin	Eijkman test (ox tallow)	Sierra test			
			'Tween 20'	'Tween 40'	'Tween 60'	'Tween 80'
Cl. histolyticum 1	*f*	−	−	−	−	−
Cl. histolyticum 2	*f*	−	−	−	−	−
Cl. multifermentans	*d*	−	−	−	−	−
Cl. fallans	*e*	−	−	−	−	−
Cl. butyricum OHII	*c, d*	−	−	−	−	−
Cl. butyricum	*c, d*	−	−	−	−	−
Cl. botulinum A. 3805	*c*	+ (4)	+ (4)	+ (4)	+ (4)	+ (4)
Cl. botulinum C. 3814	*c*	+ (4)	+ (8)	+ (8)	+ (8)	+ (8)
Cl. botolinum D. 33	*c*	+ (8)	+ (8)	+ (8)	+ (8)	+ (8)
Cl. oedematiens	*d*	+ (10)	+ (10)	+ (10)	+ (10)	+ (10)
Cl. botulinum A. 7948	*b*	+ (4)	+ (4)	+ (4)	+ (4)	−
Cl. botulinum E. 9564	*b*	+ (8)	+ (8)	+ (8)	+ (8)	−
Cl. botulinum B. 7949	*b*	+ (4)	−	+ (4)	+ (4)	−
Cl. botulinum D. 9633	*b*	+ (8)	−	+ (8)	+ (8)	−
Cl. perfringes A.	*d*	+ (8)	−	+ (8)	+ (8)	−
Cl. welchii A.	*a*	+ (10)	−	+ (10)	+ (10)	−
Cl. welchii B. 6121	*a*	+ (10)	−	+ (10)	+ (10)	−
Cl. botulinum C. 6060	*b*	+ (8)	+ (10)	−	−	−
Cl. agni C. var. *paludis*	*d*	+ (10)	−	+ (30)	−	−
Cl. agni D. var. *wilsdoni*	*d*	+ (10)	−	+ (10)	−	−
Cl. botulinum B. 213	*c*	−	+ (4)	+ (4)	+ (4)	+ (4)
Cl. sordelli	*e*	−	+ (10)	+ (10)	+ (10)	−
Staphylococcus anaerobius	*c*	−	+ (30)	+ (30)	+ (30)	−
Corynebacterium liquefaciens	*c*	−	+ (30)	+ (30)	+ (30)	−
Cl. welchii C. 4989	*a*	−	−	+ (30)	+ (10)	−
Cl. sporogenes	*d*	−	−	+ (10)	+ (10)	−
Cl. tetani Y.	*d*	−	−	+ (30)	+ (30)	−
Cl. tetani P	*d*	−	−	−	+ (30)	−

a, National Collection of Type Cultures, Central Public Health Laboratory, London; *b*, Central Hygiene Institute, Ankara; *c*, Pasteur Institute, Lille; *d*, Pasteur Institute, Paris; *e*, Wellcome Research Laboratories, Langley Court, Kent; *f*, Ibys Institute, Madrid.

or on plates in Brewer's jars. Simultaneously, lipolytic activity was tested by the Eijkman[2] method using the Prévot medium.

Table 1 shows that, of the twenty-eight strains tested, twenty-two had lipolytic activity. Although four of the lipolytic species did not exhibit any specificity, hydrolysing all four 'Tweens', eleven strains were able to hydrolyse only two or three of the four 'Tweens' tested. Four strains show a higher substrate specificity hydrolysing only one of the four 'Tweens'. Another striking feature of the 'Tween' test is its greater sensitivity than the Eijkman test. Seven strains which were non-lipolytic on the Eijkman plates were able to hydrolyse 'Tweens'.

These results may indicate that we are dealing with adaptive lipases which, however, cannot be detected with the Eijkman test (cf. ref. 1). Further investigations are being made to elucidate the specificity and mechanism of adaptation of these enzymes.

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Greater Susceptibility to *Mycobacterium johnei* of *C.57* Black Mice, when compared with *CBA* and Swiss White Mice

RESEARCH on Johne's disease, a fatal enteritis of cattle and sheep due to infection with *Mycobacterium johnei*, has long been hampered by the lack of a suitable laboratory animal as an experimental host. In more recent years, Francis[1] and Lominski, Cameron and Roberts[2] have described the experimental infection of white mice; Ford[3], however, failed to demonstrate intestinal lesions in the latter species and I have obtained intestinal lesions in only a minority of inoculated Swiss white mice (unpublished work). It was decided to compare the susceptibility of two further strains of mice with that of the Swiss white strain.

Equal numbers of *C.*57 black mice, *CBA* brindle mice (the foundation mice of both these stocks were obtained from the Medical Research Council Radiobiological Unit, Harwell, by courtesy of Dr. M. F. Lyon) and Swiss white mice (from stock maintained at this Station for several years) were inoculated intraperitoneally with 2 mgm. of moist *M. johnei* in suspension in saline; this strain of *M. johnei* was originally isolated from a case of the disease in a cow and was cultured on Taylor's medium[4].

Three months after infection, 15 mice of each strain were killed, and *M. johnei* infection of the intestines was detected, by histological methods, in two of the *C.*57 mice and one of the Swiss white mice.

Six months after infection, six mice of each strain were killed; intestinal infection was detected in all six of the *C.*57 mice and in three of the white mice, but not in the *CBA* mice.

Nine months after infection, six mice of each strain were killed. The small intestines of all six of the *C.*57 mice were visibly enlarged, due to thickening of the mucosa; histological and smear examinations of the intestines confirmed the presence of *M. johnei* in the six *C.*57 mice and in one of the six white mice, but in none of the *CBA* mice.

One year after infection, six mice of each strain were killed; the intestines of all six *C.*57 mice, and two of the six white mice, were visibly thickened. *M. johnei* infection of the intestines was detected in the six *C.*57 mice, in one white mouse and in one *CBA* mouse.

These results indicate that the three strains of mice differ in their susceptibility to intestinal infection with *M. johnei*. The susceptibility of the *C*.57 black mice was markedly higher than that of the other strains.

A full report of this experiment will be published elsewhere. Further experiments are in progress to ascertain the suitability of the *C*.57 black mouse as a laboratory animal for work on Johne's disease.

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The Host-Range of a *Streptomyces aureofaciens* Actinophage

A VIRULENT actinophage destructive to *S. aureofaciens* cultures and chlortetracycline (the trade mark of American Cyanamid Company for chlortetracycline is 'Aureomycin') fermentations had previously been reported[1]. Limited data regarding its host-range obtained at that time showed that it was a monovalent phage. Since other actinophages have been noted to be either polyvalent or monovalent[2], further tests have been made with the *S. aureofaciens* phage to establish more clearly the nature of its host-range.

Evidence is presented in this communication confirming that this phage is species-specific for *S. aureofaciens*. Tests for susceptibility to *S. aureofaciens* phage were made by spreading *Streptomyces* spore suspensions over the surface of corn-steep agar, resulting in confluent growth surfaces within 24–36 hr. When the spore suspensions had dried, the seeded surfaces were spotted with drops (0·01–0·02 ml.) of phage suspension using two concentrations in broth, 3×10^9 and 3×10^8 phage particles per ml. Controls consisted of broth and water drops spotted on the seeded surfaces. Observations for the presence of plaques were made after 1, 2, and 4–5 days. All tests were made at least in duplicate; in the case of a doubtful reaction, tests were repeated.

Representatives of forty diverse named species of *Streptomyces* other than *S. aureofaciens*, selected to encompass most sections and series of the genus as set up by Pridham *et al.*[3], showed no reaction to the phage. Tests were made with 3–5 strains each of *S. griseus*, *S. lavendulae*, *S. fradiae*, *S. antibioticus*, *S. rimosus*, *S. hygroscopicus*, *S. purpurascens*, *S. albogriseolus*, *S. tanashiensis* and *S. erythreus* and with one strain each of 30 additional species, use being made wherever possible of authentic strains from type culture collections.

Twenty-five soil isolates determined to be *S. aureofaciens* on the basis of morphological, cultural and physiological characteristics were also used in this work. Eighteen of these isolates were susceptible to the virulent *S. aureofaciens* phage, though some less than others, as indicated by reduced plating efficiency. The seven resistant isolates gave indications that their resistance was connected with the presence of homologous temperate phage(s) in their cultures. It is not definitely known whether these cultures are lysogenic or carry phage. Results with the *S. aureofaciens* group of isolates are recorded in Table 1.

Table 1. RESPONSE OF *Streptomyces aureofaciens* STRAINS TO *S. aureofaciens* PHAGE

Collection* designation	Phage reaction†	Collection* designation	Phage reaction†
A-1787	++	*AB*-357	−
A-2021	−	*AB*-708	+++
A-2380	++	*AC*-105	++
A-4342	+++	*AD*-417	−
A-5156	+++	*T*-2146	+
A-6496	+++	*T*-3452	±
A-7189	+++	*T*-3491	−
A-7300	±	NRRL 2209	+++
A-8202	−	NRRL *B*-1286	+++
A-9635	+++	NRRL *B*-1287	−
AA-689	+	NRRL *B*-1288	++
AA-772	++	ATCC 12416	+
AB-46	−		

* The designations of the first 20 strains are those of the Lederle Collection.

† Phage susceptibility (as indicated by plating efficiency): +++, high; ++, intermediate; +, low; ±, very low (a few plaques observed); −, none (no plaques observed).

The possible utility of actinophage susceptibility as a criterion for species differentiation in the genus *Streptomyces* has been the subject of much discussion among investigators concerned with the taxonomy of this group. It seems to be generally agreed that polyvalent phages would be difficult to utilize because of their complex host relationships, but that species-specific, monovalent phages would have definite applicability, in conjunction with other taxonomic features characterizing *Streptomyces* species[2,4,5]. Susceptibility of newly isolated *Streptomyces* cultures to such a species-specific phage would reinforce other results on their close relationship to the phages' host species. Lack of susceptibility, however, would not exclude an isolate from being allied with the host species, as species susceptible to phages are generally known to comprise strains resistant to the same phages.

In the light of this evidence that the subject *S. aureofaciens* actinophage is species-specific, it was extremely interesting from a taxonomic point of view to extend this investigation to include three controversial organisms, *S. viridifaciens*[6], *S. psammoticus* (*S. feofaciens*)[7] and *S. sayamaensis*[8]. Although described as separate new species by those who isolated them, these organisms have morphological, cultural and physiological properties which raise serious doubts as to their distinction from *S. aureofaciens*. The results obtained, all three proving to be susceptible to the virulent, monovalent *S. aureofaciens* phage, add support to the contention that these three species should be reduced to synonymy with *S. aureofaciens*.

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GENETICS

Morphological Separation of Cytological Races in *Ranunculus ficaria* L.

THE presence of axillary tubercles ('bulbils') in the various races of *Ranunculus ficaria* L. *sensu lato* is still a matter of some debate. So far as British material is concerned, previous workers have shown that all tubercle-bearing plants investigated cytologically have been tetraploid, with $2n = 32$, while non-tubercle-bearing plants have been diploid ($2n = 16$). Marsden-Jones[1] proposed separating the tubercle-bearing plants as var. *bulbifera*, as opposed to typical non-tubercle-bearing plants which were later described by Clapham[2] as var. *fertilis*. The two races were later raised to sub-specific rank by Lawalrée[3].

Other differences between the two races given by Marsden-Jones were: number and relative width of the petals, range in stamen and carpel number, proportion of viable pollen and viable seed set. This range of variation in stamens and carpels is such, however, that they do not form useful characters for taxonomic separation.

Presence or absence of bulbils has always been considered a distinguishing character for the two races. However, it has not always been realized that the tubercles are only produced towards the end of the flowering season and this in itself has caused some confusion: presence of tubercles is a positive character while absence may imply true absence or simply young material which will later develop tubercles. Almost equal reliance has been placed on the suggested floral differences for taxonomic distinction, notably petal number and degree of overlap, particularly in the absence of tubercles. These differences have been widely accepted and never, as far as we are aware, disputed.

Field and laboratory studies on local populations in north Wales and Cheshire during the past few years have pointed to the unreliability of these floral characters as a means of distinguishing the two cytological races. In some populations the characters given by Marsden-Jones hold good but it is not uncommon to find them completely reversed in other populations. This is specially true of the petal characters. Thus while it is possible to separate populations in the same locality into morphologically different types, agreeing with the diploid and tetraploid races suggested by Marsden-Jones, actual cytological investigation has shown that some apparent tetraploids have proved to be diploids and vice versa.

In Table 1 details are given of the floral characters and cytological status of a selection of the populations studied in north Wales and Cheshire. Measurements were made of samples of 100 plants in each population. The following points may be noted.

Petal range. Although the mean petal number has usually been found to differ between diploid and tetraploid populations, the position of the mean is not correlated overall with the level of ploidy (for example, Nos. 5906, 5915 in Table 1); similarly, although there appears to be a difference in petal range this is not constant and in any event the overlap between the two makes it an unreliable character.

Petal type. Two types of petal are commonly recognized: broad and overlapping in the diploid and narrow, not overlapping, in the tetraploid. In practice we have found an intermediate to occur so frequently that it became necessary to recognize a third type. This intermediate type has been found in both races. Apart from this, the broad-type petal has been found in tetraploids and the narrow in diploids, contrary to what is normally stated (Nos. 5906, 6001). Consequently, no reliance can be placed on petal type as a diagnostic character.

Table 1

Reference No.	Character						
	Axillary tubercles + or −	No. of stamens	No. of carpels	No. of petals	Petal type	Chromosome number $n =$	Habitat
5903	−	27–35	25–44	(7–)8(–11)	1	8	Hedgerow
5904	−	19–37	22–31	8(–10)	2	8	Hedgerow
5906	−	50–60	45–50	9–11	3	8	Woodland
5907	+	30	30	.	1	16	Hedgerow
5908	−	24–43	26–45	8(–9)	2	8	Roadside
5909	−	23–30	14–37	(7–)8(–9)	1	8	Roadside
5910	−	30	30	8	1	8	Woodland
5912	+	35	30	.	3	16	Woodland, riverside
5915	+	20	12–19	7–8	2	16	Riverside
5916	−	17–40	13–30	8 (–10)	2	8	Woodland
5917	+	23–30	23–30	7–8	3	16	Woodland
6001	+	22–32	7–26	8–10	1	16	Woodland
6002	−	23–29	13–23	8	3	8	Woodland

+ or − is presence or absence of axillary tubercles. Petal type: 1, broad, overlapping; 2, intermediate; 3, narrow, not overlapping.

The conclusions that may be drawn from the above data are that in the absence of axillary tubercles, in young material, petal type is not a reliable cytological marker since it is not constantly correlated with level of ploidy. Furthermore, it is not entirely certain that the association of tubercles with the tetraploid is always constant, although it would generally appear to hold good. It may be possible to separate the diploids and tetraploids by other characters associated with the frequency and position of the flower buds in the inflorescence, but in any event it is quite clear that the recognition of the different cytological races by morphological characters is by no means so simple as is usually believed, if indeed it is consistently possible at all. It should be emphasized that these studies have been made on populations from only a small part of the range of the species and they are being extended to other areas. Details of the present and other investigations will be published separately.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, February 20

University of London (at the Institute of Education, Malet Street, London, W.C.1), at 5.15 p.m.—Prof. J. W. Tibble: "The Training College and Higher Education."*

Society of Chemical Industry, Pesticides Group (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Prof. T. A. Bennet-Clark, F.R.S.: "Experimental Design in Pesticide Research".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. H. B. G. Robinson (Kansas City): "Some Effects of Ageing on the Oral Structures".*

Institution of Mechanical Engineers, Nuclear Energy Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Influence of Availability of Enrichment upon Reactor Type and Design".

Institution of Electrical Engineers, London Graduate and Student Section (at Savoy Place, London, W.C.2), at 6.30 p.m.—Mr. K. M. Jones: "Testing High Voltage Interconnected Systems with particular reference to the Kariba Scheme".

Tuesday, February 21

University of London (at the Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 1.30 p.m.—Mr. J. Christopher Jones: "Human Factors in Engineering Design".*

Institution of Electrical Engineers, Measurement, Supply and Utilization Sections (at Savoy Place, London, W.C.2), at 5.30 p.m.—Scientific Papers.

Royal Society of Medicine, Pathology Section (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Meeting on "Electron Microscopy".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. R. W. Riddell: "Fungi Causing and Complicating Human Disease".* (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. K. H Spring: "Introductory Survey".* (First of five lectures on "Generation of Electricity without the use of Rotating Machinery".)

Institute of Information Scientists (at the Caxton Hall, Caxton Street, London, S.W.1), at 6.15 p.m.—Dr. J. G. Malloch: "The Work of the British Commonwealth Scientific Organization".*

Society of Chemical Industry, Plastics and Polymer Group (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. D. C. Libby: "Magnetic Resonance Studies on High Polymers".

Royal Aeronautical Society (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. N. P. Inglis: "Newer Metallic Materials of Construction".

Wednesday, February 22

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. J. F. Loutit: "Radiation and Leukæmia".*

Institute of Physics and the Physical Society, Colour Group (at Battersea College of Technology, Battersea Park Road, London, S.W.11), at 3 p.m.—Dr. V. S. Griffiths: "Colour and Chemistry".

Institution of Electrical Engineers, Electronics and Communications Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. J. M. Meggitt: "Coding Theory and its Applications".

British Institution of Radio Engineers, Medical Electronics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Discussion on "Transistorized Medical and Biological Electronic Equipment".

Institution of Mechanical Engineers (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Prof. P. M. S. Blackett, F.R.S.: "Controlled Fusion Reactions" (Thomas Hawksley Lecture).

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—The Right Hon. Lord Nathan, P.C.: "Geography and the Business World To-day".

Society of Chemical Industry, Food and Microbiology Groups (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "The Microbiology of the Deep Frozen Foods". Dr. H. Rachel White. "Some Aspects of the Microbiology of Frozen Peas"; Dr. B. J. Wagg and Miss M. Boothroyd: "The Effect of Processing and Freezing on the Bacteria in Frozen Meat Products".

British Psychological Society, Social Psychology Section (in Room 425, Birkbeck College, Malet Street, London, W.C.1), at 8 p.m.—Section Annual General Meeting. Dr. W. A. Belson. "Television and the Family".

Thursday, February 23

Chemical Society (in the Large Chemistry Lecture Theatre, Imperial College of Science and Technology, London, S.W.7), at 10.30 a.m.—Symposium on "Terpene Chemistry".

University of London (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. W. R. G. Horton: "Gods as Guests—Ritual Drama in a West African Tribe".*

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Sir Gordon Sutherland, F.R.S.: "Some Recent Developments at the National Physical Laboratory".

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. H. J. Koch: "Metabolism and Variation in the Blind Cave Fish *Caecobarbus geertsi*"; Dr. V. C. Robertson: "The Jebel Marra, Western Sudan".

University of London (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Dr. W. J. Whelen. "Recent Advances in the Chemistry and Biochemistry of Glycogen and Starch".* (Further lecture on March 2.)

University of London (at Guy's Hospital Medical School, St. Thomas's Street, London, S.E.1), at 5.30 p.m.—Dr. Morton I. Grossman (Veterans Administration Centre, Los Angeles): "Regulation of Pancreatic Secretion".*

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. G. B. Koelle (University of Pennsylvania): "Acetylcholinesterase in Synaptic and Neuroeffector Transmission".*

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. G. Everson Pearse: "Enzyme Histochemistry and its Biochemical Alignments".* (Thirteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institute of Metals, Metal Physics Committee (at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Prof. W. Hume-Rothery, F.R.S.: "The Theory of the Alloys of Copper".

Oil and Colour Chemists' Association (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. N. R. Barucha: "Pigments in Corrosion Prevention".

Friday, February 24

British Psychological Society, Occupational Psychology Section (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Mr. J. D. Handyside: "Satisfactions and Aspirations" (Chairman's Address).

Institute of Rural Life at Home and Overseas (at Friends International Centre, 32 Tavistock Square, London, W.C.1), at 1.15 p.m.—Commissioner H. A. Lord: "The Needs of Rural People in Korea".*

University of London (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. H. Harris. "The Inborn Errors of Metabolism".*

Royal Astronomical Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Radio Studies of the Aurora". Chairman: Mr. J. A. Ratcliffe, F.R.S. Speakers: Dr. J. W. Dungey, Dr. S. Evans, Dr. C. D. Watkins and Mr. W. R. Piggott.*

Society of Analytical Chemistry, Microchemistry Group (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.45 p.m.—Annual General Meeting. 7 p.m.—Mr. F. Holmes: Retiring Chairman's Address.

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Martin Ryle, F.R.S.: "Radio Astronomy and Cosmology".

Saturday, February 25

Biochemical Society (in the Physics Lecture Theatre, Imperial College of Science and Technology, South Kensington, London, S.W.7), at 10.10 a.m.—403rd Meeting.

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. Robinson: "Bird Watching in the Medway Valley".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Chemical Research Assistant/Technician (preferably with experience of steroid chemistry) to work with a team investigating aspects of chemical endocrinology in relation to human biology and genetics—Dr. J. M. Tanner, Institute of Child Health, Great Ormond Street, London, W.C.1 (February 23).

Assistant Lecturer or Lecturer in Physics—The Registrar, The University, Sheffield (February 25).

Assistant Lecturer or Lecturer (with a special interest in physiology) in Botany—The Secretary, The University, Exeter (February 25).

Junior Lecturer (preferably with interests in the fields of either (*a*) soils and climate or (*b*) regional geography) in Geography—The Registrar, Trinity College, Dublin, Republic of Ireland (February 27).

Research Student (with experience in one of the following fields medicine, chemistry, biochemistry, or pharmacology) in Pharmacology, for work on naturally occurring neuro-muscular blocking agents—The Registrar, Trinity College, Dublin, Republic of Ireland (February 27)

Assistant Lecturer (with a good honours degree with special interests in mineralogy) in Geology—The Registrar, King's College (University of London), Strand, London, W.C.2 (February 28).

Lecturer or Assistant Lecturer in the Department of Mathematics, Makerere College, The University College of East

Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 28).

UNIVERSITY LIBRARIAN—The Secretary and Registrar, The University, Southampton (February 28).

PLANT PHYSIOLOGIST (with a good honours degree in botany and research experience) for investigations of effects of temperature, oxygen and carbon dioxide on the respiration of fruit—The Director, Ditton Laboratory, A.R.C., Larkfield, Maidstone, Kent (March 1).

RESEARCH ASSISTANT to operate and maintain the electron microscope and ancillary equipment in the Metal Physics Section of the Cavendish Laboratory—The Secretary of the Department of Physics, Cavendish Laboratory, The University, Free School Lane, Cambridge (March 1).

EDUCATION PSYCHOLOGIST, Grade I (man or woman)—The Director of Education, Shire Hall, Durham (March 3).

LECTURER (with a good honours degree, teaching experience up to degree level, and research experience) IN ZOOLOGY at the Nigerian College of Arts, Science and Technology, Zaria Branch—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (March 3).

LECTURER (preferably with a special interest in microwaves and microwave devices, both vacuum and solid state) IN APPLIED PHYSICS—The Secretary, The University, 38 North Bailey, Durham (March 4).

RESEARCH ASSISTANT (British nationality) IN THE WHEATSTONE LABORATORY for research on cathodoluminescence, scintillation properties, etc., of certain elementary crystals—Prof. F. C. Champion, Department of Physics, King's College (University of London), Strand, W.C.2 (March 5).

LIBRARIAN at the University College (Fourah Bay College), Sierra Leone—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 6).

HELMINTHOLOGIST, SCIENTIFIC ASSISTANT (with a degree and experience in helminthology) to prepare abstracts and assist in editorial work on *Helminthological Abstracts*—The Acting Director, Commonwealth Bureau of Helminthology, The White House, 103 St. Peter's Street, St. Albans, Herts (March 11).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN BIOCHEMISTRY, CHEMISTRY, CHEMICAL OCEANOGRAPHY, ENGINEERING and PHYSICS—The Registrar, The University, Southampton (March 11).

LECTURER IN PHYSICAL and/or INORGANIC CHEMISTRY in St. Salvator's College, St. Andrews—Joint Clerk to the University Court, The University, St. Andrews (March 11).

OFFICIAL FELLOW and LECTURER IN PHYSIOLOGY; and a DEMONSTRATOR IN PHYSIOLOGY—The Warden, New College, Oxford (March 11).

UNIVERSITY DEMONSTRATOR IN PHYSIOLOGY—The Administrator, Department of Physiology, The University, South Parks Road, Oxford (March 11).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (March 13).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY—The Registrar (Room 22, O.R.B.), The University, Reading (March 18).

LECTURER IN GEOGRAPHY at Rhodes University, Port Elizabeth, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa and London, March 20).

READER IN INORGANIC CHEMISTRY at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (March 20).

SENIOR LECTURER IN HORTICULTURE at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 25).

SENIOR LECTURER IN PHYSICS at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 28).

FERRANTI FELLOW (with at least two years research experience) IN COMPUTING, to do original work in any field connected with computation or other application of digital computers, with access to a Ferranti Mercury—The Director, University Computing Laboratory, 9 South Parks Road, Oxford (July 31).

DEMONSTRATOR IN AGRICULTURAL BOTANY; and a DEMONSTRATOR IN AGRICULTURAL ZOOLOGY at the School of Agriculture, Sutton Bonington—The Registrar, The University, Nottingham.

JUNIOR RESEARCH FELLOW IN CHEMISTRY—The College Secretary, Balliol College, Oxford.

LECTURER (preferably with industrial experience such as in nuclear fuel processing) IN CHEMICAL ENGINEERING for the Nuclear Technology Group—G. R. Hall, Department of Chemical Engineering and Chemical Technology, Imperial College of Science and Technology, London, S.W.7.

LECTURER (with a good medical qualification and some laboratory experience) IN THE DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY—The Dean, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1.

TECHNICIAN (female) IN THE PHYSICS LABORATORY, for work concerned chiefly with diagnosis and treatment using radioactive isotopes—The Hospital Secretary, Dudley Road Hospital, Birmingham, 18.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Association of Teachers in Technical Institutions. Comments on the Crowther Report. Pp. 15. (London: Association of Teachers in Technical Institutions, 1960.) [1711

The Chest and Heart Association. Smoking: a Message from an M.O.H. Pp. 8. (London: Chest and Heart Association, 1960.) [1711

Annual Report and Accounts of the British Broadcasting Corporation, 1959–60. Pp. 175+8 plates. (Cmnd. 1174.) (London: H.M. Stationery Office, 1960.) 9*s*. net. [1711

Symposia of the Zoological Society of London. No. 3: Sensory Specialization in Response to Environment Demands—Proceedings of a Symposium held at the Zoological Society of London, Regent's Park, London, on 4th March, 1960. Pp. iv+83+3 plates. (London: Zoological Society of London, 1960.) 17*s*. 6*d*. [1711

Forestry Commission. Bulletin No. 32: Afforestation of Upland Heaths. By J. W. L. Zehetmayr. Pp. v+145+34 plates. (London: H.M. Stationery Office, 1960.) 17*s*. 6*d*. net. [1711

Titles of Dissertations approved for the Ph.D., M.Sc., and M.Litt. Degrees in the University of Cambridge during the Academical Year 1959–60. Pp. iii+20. (Cambridge: Board of Research Studies, The University, 1960.) [1711

University of Leeds. Annual Report, 1959–60 (Draft of principal sections). Pp. ii+37. (Leeds: The University, 1960.) [1711

National Physical Laboratory. Effects of Variation in the Ambient Air on the Calibration and Use of Ionization Dosemeters. By Dr. G. P. Barnard, Dr. G. H. Aston and A. R. S. Marsh. Pp. 31. (London: H.M. Stationery Office, 1960.) 2*s*. 6*d*. net. [1811

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1025, Vol. 253 (17 November, 1960): The Classical Field Theory of Matter and Electricity. 1: An Approach from First Principles. 2: The Electromagnetic Theory of Particles. By S. R. Milner. Pp. 185–226. 13*s*. 6*d*. No. 1026, Vol. 253 (19 November, 1960): Quadratic Diophantine Equations. By G. L. Watson. Pp. 227–254. 9*s*. No. 1027, Vol. 253 (17 November, 1960): On Adsorption at the Oil/Water Interface and the Calculation of Electrical Potentials in the Aqueous Surface Phase. 2: The Effects of Ionic Size. By D. A. Haydon and F. H. Taylor Pp. 255–275. 6*s*. 6*d*. (London: The Royal Society, 1960.) [1811

Other Countries

United States Department of the Interior: Geological Survey, Bulletin 1045-F: Core Logs from Five Test Holes near Kramer, California. By William K. Benda, R. C. Erd, and Ward C. Smith. Pp. iv+319–393+plates 11 and 12. Bulletin 1085–C: Botanical Prospecting for Uranium in the Circle Cliffs Area, Garfield County, Utah. By Frank J. Kleinhampl and Carl Koteff. Pp. iii+85–104+plates 7 and 8. Bulletin 1104–A: Erosion and Related Phenomena at Paricutin in 1957. By Kenneth Segerstrom. Pp. iii+18+1 plate. 50 cents. Bulletin 1111–A: Devonian Rugose Corals from Northern Maine. By William A. Oliver, Jr. Pp. iii+23+plates 1–5. Bulletin 1116–A: Geophysical Abstracts, No. 180, January-March, 1960. By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel, and others. Pp. iii+128. 40 cents. (Washington, D.C.: Government Printing Office, 1960.) [1711

United States Department of the Interior: Geological Survey. Professional Paper 260–X: Smaller Foraminifera from Eniwetok Drill Holes. By Ruth Todd and Doris Low. Pp. iv+799–861+plates 255–264. Professional Paper 260-Y: Drilling Operations on Eniwetok Atoll. By Harry S. Ladd and Seymour O. Schlanger. Pp. iv+863–903+plates 265 and 266. Professional Paper 282-D: Flow Resistance in Sinuous or Irregular Channels. By Luna B. Leopold, Ralph A. Bagnold, Mr. Gordon Wolman and Lucien M. Brish, Jr. Pp. iv+111–134+plates 3 and 4. Professional Paper 282-E: Some Aspects of the Shape of River Meanders. By Ralph A. Bagnold. Pp. iv+135–144. 20 cents. Professional Paper 315–C: Stratigraphic Distribution of some Pennsylvanian Fusulinidae from Brown and Coleman Counties, Texas. By Donald A. Myers. Pp. iii+37–53+plates 15–24. 45 cents. Professional Paper 316-D: Gravity Survey of the Western Mojave Desert, California. By Don R. Mabey. Pp. iii+51–73+plates 10–12. (Washington, D.C.: Government Printing Office, 1960.) [1711

United States Department of the Interior: Geological Survey. Professional Paper 334-F: Ammonites of Early Cretaceous Age (Valanginian and Hauterivian) from the Pacific Coast States. By Ralph W. Imlay. Pp. iii+167–228+plates 24–43. 1.25 dollars. Professional Paper 340: Trepostomatous Bryozoa of the Hamilton Group of New York State. By Richard S. Boardman. Pp. iii+87+plates 1–22. 1.25 dollars. Professional Paper 352-B: The Shape of Alluvial Channels in Relation to Sediment Type. Pp. iii+17–30+plate 5. Professional Paper 354-D: Early Cretaceous (Albian) Ammonites from the Chitina Valley and Talkeetna Mountains, Alaska. By Ralph W. Imlay. Pp. iii+87–114+plates 11–19. 50 cents. Professional Paper 354-G. Deposition of Uranium in Salt-Pan Basins. By Kenneth G. Bell. Pp. iii+161–169. 20 cents. Professional Paper 400-B: Short Papers in the Geological Sciences—Geological Survey Research 1960. Pp. viii+515. 4.25 dollars. (Washington, D.C.. Government Printing Office, 1960.) [1711

The Weizmann Institute of Science. Serial Holdings of the Weizmann Institute of Science (complete until May, 1960). Pp. iii+62. (Rehovoth, Israel: Weizmann Institute of Science, 1960) [1711

Queensland. Fifteenth Annual Report of the Council of the Queensland Institute of Medical Research, year ended 30th June, 1960. Pp. 26. (Brisbane: Queensland Institute of Medical Research, 1960.) [1711

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SCIENTIFIC AND TECHNICAL RESOURCES OF BRITISH RAILWAYS

THE debate on British Railways in the House of Commons on October 26, which was opened by the Minister of Transport, Mr. E. Marples, and that in the House of Lords on problems of transportation on December 7, were both prompted essentially by the annual report and accounts of the British Transport Commission for the year ended December 1, 1959, and by the report from the Select Committee on Nationalized Industries dealing with British Railways*. The latter report is of considerable scientific interest, particularly those sections dealing with technical resources, design and research and with man-power and productivity. It includes a memorandum by the British Transport Commission on research and other memoranda on man-power, productivity and training and on qualified scientists and engineers—memoranda which are of greater interest in view of the observations on research and development in the chapters which Prof. W. A. Robson devotes to those subjects in his recent book on *Nationalized Industry and Public Ownership*.

Some of the realism was taken out of both debates by Mr. Marples's announcement at the start that the Government was also considering the recommendations of a Special Advisory Group under Sir Ivan Stedeford, which is supplementing the work of the Select Committee, and that it intended to place its proposals before the House as soon as possible in the form of a comprehensive paper. Nevertheless, this can scarcely explain the absence of reference to these vital aspects of the railway services in both debates The Minister for Science made no reference to research or to scientific or technical staff in replying on the debate on December 7, although he agreed to the need for an efficient and modernized railway system as far ahead as could be seen. Only by Mr. Austen Albu was any real emphasis placed on these factors or indeed any reference made.

Mr. Albu, himself a member of the Select Committee, asserted that modern industry needs a scientific spirit throughout its whole organization and not only among its technical staff; he suggested the appointment as general managers on the Railways of one or two engineers with a modern university background. Meanwhile, he thought the Transport Commission should consider how to achieve greater co-ordination between the various branches of its engineering staff. He pointed out that the railway systems of the world, including the British railway system, have never done much research, and that the Transport Commission is only now beginning to build up a proper research staff. Although the industry is undergoing rapid technological change, only 0·3 per cent of its employees are qualified scientists or engineers; between 1956 and 1959 the number of professionally qualified engineers decreased from 598 to 484. In 1959 only 277 qualified electrical engineers were employed, and 512 graduate scientists and engineers. Witnesses before the Select Committee showed no awareness at all of the level of professional staff required to deal with the revolutionary changes in progress.

Mr. Albu's alarm at this position, his concern at the relatively low level of salaries offered to engineers by the Transport Commission, and his assertion that the Commission has not begun to understand the revolution in education in Britain since the end of the War, was endorsed by Sir Toby Low, chairman of the Select Committee; but his points were not developed further in the debate, which was concerned excessively with political issues, nor were they considered by Mr. John Hay, Joint Parliamentary Secretary to the Ministry of Transport, in replying on the debate. Prof. Robson devoted some attention to research in nationalized industry generally in his book, but was concerned chiefly with the organization of research both within such industries and within the Government; and while agreeing to the need for research in these industries, he gave no indication that he had modified the opinion expressed earlier in *Problems of Nationalized Industry*, that Britain should not rely excessively on these industries to conduct more fundamental scientific research.

Since that opinion was expressed in 1952, the Herbert Committee has reported adversely on the extent to which the electricity supply industry leans on research efforts by outside organizations and its failure to devote sufficient resources to the planning of the future. The surveys of scientific and engineering man-power in Great Britain made for the Advisory Council on Scientific Policy by its Committee on Scientific Man-power have shown how poorly the research effort of the nationalized industries, in terms of man-power, apart from the Atomic Energy Authority, compares with that of industry in general; and in its last annual report the Advisory Council on Scientific Policy estimated expenditure on research and development in the United Kingdom by the public corporations at £6·9 million in a total of £477·8 million. The British Transport Commission in 1956 and in 1959 employed a smaller percentage of scientists and engineers in proportion to the total number of workers employed in the industry than in any other nationalized industry (0·25 in 1959 compared with 0·9 in the Airways Corporations and 0·8 in the National Coal Board).

These circumstances alone give special interest to the Select Committee's examination of the adequacy of the research organization and programme of

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* Report from the Select Committee on Nationalized Industries, together with the Proceedings of the Committee, Minutes of Evidence and Appendices—British Railways. Pp. cvi+505. (London: H.M. Stationery Office, 1960.) 22s. 6d. net.

British Railways. The memorandum submitted to the Committee by the Commission emphasizes that the Commission regards its primary function as the provision of a transport service, and, as its suppliers undertake research and development, it has relied to a large extent on these suppliers for research, although it appreciates that in certain fields it must conduct its own research. As examples, the memorandum quotes problems concerning the interaction of vehicle and track, stresses in rails, corrosion, maintenance troubles, the interaction between equipment supplied by different manufacturers and field-trials on all types of transport. The total annual cost of identifiable research and development within the Commission is estimated at £2 million, of which some £700,000 is for research, 90 per cent being for the Railways, according to Sir Brian Robertson. Much of this, however, is for day-to-day advice and routine investigations. The research organization has been built up on lines recommended by a committee of scientists in November 1948. It provides for a technical committee and a research advisory council; a chief of research, assisted by a research co-ordination committee and with a small research department at headquarters; separate research departments for British Railways and Docks and Waterways, and for London Transport; and for electric traction research and operational research. The memorandum emphasizes the contact maintained with the Department of Scientific and Industrial Research and other Government departments, with the National Research Development Corporation, its membership of some thirty research associations and its contribution to the Office for Research and Experiments set up by the International Union of Railways to undertake co-operative research on common and technical problems and to pool the means of technical research.

In examination before the Committee, Sir Brian Robertson agreed that far too little was being spent on research, but he directed attention to adverse criticism which had been incurred when the Commission extended its facilities by building a small laboratory at Muswell Hill for servicing the Southern Region. Railway systems all over the world need laboratories and facilities for research, and it is obvious from Sir Brian's remarks that an increase in public understanding is essential if research activities are to be extended and strengthened. He referred particularly to the problems arising in the contact of rail and wheel as requiring fundamental research, but claimed that the Commission has itself stimulated some fundamental research by its suppliers. British Railways is at present engaged in fundamental measurements of currents generated at various parts of the electrification system, currents generated in cables alongside and mechanical measurements of the movements of vehicles in service on its new 25-kV. system. The new laboratory projected at Derby, where the expanded research department is to be located, would increase the annual expenditure on research by about £130,000 to £150,000; and the memorandum submitted to the Select Committee points out that the scientific background to the dynamic problems involved in the introduction of long welded rail is still very obscure and difficult, and, a long-term research project dealing with this is being assisted by one of the universities. There is also a special panel concerned with permanent-way problems which constitute the more important research projects.

Little emerged in the evidence before the Select Committee to indicate the magnitude of any deficiency in the research effort of British Railways; but although the Transport Commission admits that expenditure on research is too low and is building up its resources, every plea for increased expenditure on research comes under critical scrutiny both of the Government and of the Press, and, like most expenditure on research, is difficult to justify on purely economic grounds. The Select Committee accepted the contention that a comparison with sums spent by other industries is not valid because railways are not a production industry; it is a question rather of seeing what needs to be done and then doing it. Nor does the report offer any real criticism of the organization for research. The Research Advisory Council appears to be accepted, and the arrangements for co-ordination of research regarded as satisfactory.

It is possible that the relative expenditure on railway research in Britain is considerably less than in other countries, and the Select Committee seems satisfied that British Railways would benefit by increased expenditure on research, though it is difficult to assess how much more should be spent; the Transport Commission considers that the additional expenditure which will follow the completion of the new laboratories at Derby will bring research expenditure to the limit that its research staff can handle efficiently. Efficiency in research depends, in fact, on an adequate technical staff, and, if full value is to be obtained from increasing investment in research, it is essential that British Railways should attract larger numbers of technical staff of the highest quality. Sir Brian Robertson seems confident that the new laboratories will enable British Railways to obtain the kind of men needed, but probably the most disturbing feature of the Select Committee's whole report is the position it reveals of inadequate resources in respect of technical staff.

The memorandum on man-power, productivity and training submitted by the Transport Commission gives the number of technical staff at the end of February 1960 as about 7,700 out of a total staff of some 519,000 at the end of December 1959. Although rates of wastage have varied between 7 and 8 per cent per annum, it has been possible during the past four years to increase the number of technical staff by more than a third; but among engineering graduates, of whom only 512 were employed in 1959, the wastage may be as high as 15 per cent. A further memorandum on qualified engineers and scientists puts the total of such staff at 1,641 in 1959 compared with estimated requirements of 2,031, and difficulties are noted in recruiting qualified engineers and scientists for some departments. There are at present 52 student engineers in training in 'sandwich courses' throughout Britain, and 35 young students from the railways are attending courses in engineering at half a dozen technical colleges or colleges of technology.

These figures indicate that the total number of professionally qualified scientists and engineers employed by British Railways at the end of 1959 has returned to the 1956 level of 1,501 after falling to 1,354 earlier in 1959. Nevertheless, an increase of only 5·8 per cent has been achieved compared with the 30·9 per cent estimated in the Survey of Scientific and Engineering Man-power in 1956. Admittedly, the British Transport Commission cannot offer the attractions of an expanding industry, but the relative increase in technical staff as a whole compared with professionally qualified staff implies some progressive lowering of quality at a time then the rapid technical changes of modernization alone clearly require the reverse. This failure in recruitment may be due to some uncertainty as to the permanency of the positions which the Commission can offer, in spite of assurances given by the Commission itself; it can equally be due to the lower salaries offered in the middle range, as Mr. Albu emphasized in the House of Commons.

It is possible to make too much of the wastage of engineering graduates. This may not be a serious matter from the point of view of the nation generally. It is the practice for young engineers in the early days of their careers to change jobs frequently to gain wider experience; but for the railways this wastage does not appear to be balanced by new recruitment of experienced men, and it seems clear also that the Commission is doing less than many industrial firms to assist recruitment from universities and technical colleges by providing scholarships to such institutions. Even at the technician-level, the numbers being recruited or trained are small, and this source has not produced the number of professionally trained engineers expected.

There are some differences in the recruitment of different classes of engineers, and while the Transport Commission regards the position as reasonably satisfactory with civil and with electrical engineers, that with regard to signal engineers is very different, although there is not here the wastage experienced in other fields. Difficulties in recruitment have, in fact, been great enough to cause the Commission to rely to some extent on technicians from abroad, and the effects of this particular shortage have been very serious and have led to a slowing down of electrification schemes in general. The shortage of qualified signal engineers is admitted to be a principal factor governing the speed of modernization.

The Select Committee sums up the situation by emphasizing that although technical staff has increased rapidly in recent years, in the main it has been staff lacking in any formal qualification. Undue reliance on recruitment from craft apprentices in the workshops and the high level of wastage have been particular factors, and although the situation is serious enough to lead one to expect the Commission to keep a careful eye on the comparatively few first-class engineers it employs, this has not been done. The Select Committee is not satisfied that the Commission has treated this matter with the urgency it deserves, and expresses surprise that no central records are kept of its professionally qualified staff.

One serious consequence of this shortage of qualified technical staff has been in the field of design, where it has clearly affected the Commission's operations during the period of modernization. The costly failure in the design of vacuum brakes for mineral wagons was probably due to this, and while the Select Committee agrees with the Commission that the railways should not seek to recruit sufficient technical staff to make them wholly independent of the design and research of those privately owned firms which manufacture locomotives, rolling stock and signal equipment, expert staff is essential to the railways for specifying what is wanted and for choosing between conflicting designs. This is particularly important at a time of major technical change, and while it seems to the Select Committee that the Commission chose the right priorities in its plans for modernization, it failed to recruit the technical staff which it recognizes as necessary for their execution.

There are other shortcomings of management to which the Select Committee directs attention, some of which are also of scientific interest. This question of scientific and technical staff, however, is probably the decisive factor, and it is essential that no political or financial controversy should be allowed to push it again into the background. The Select Committee has rendered very great service in this report by bringing this issue to the forefront; and although the progress of plans for modernization may help to remove some of the misconceptions which have hindered the recruitment of qualified young men into what is thought by some to be a declining industry, there is no doubt that scientists and technologists in general could do something to combat ill-informed or prejudiced criticism—particularly of the type which has made the Commission so diffident about increasing its expenditure on research. As the Select Committee points out in its general conclusions, the scientific and technical advances which have made Britain famous in other fields have not in recent years borne full fruit in the railways; for on the evidence received, a large-scale British railway system could undoubtedly be profitable. The number of improvements in hand, and the number of points at which further improvements can be made, support this; but this is not solely a matter of efficient management and of adequate scientific and technical resources—in staff as well as in equipment and finance. It is at least in part a matter of the climate of opinion; and in creating and maintaining the climate of opinion which will support the necessary deployment of resources, scientists and technologists have an indispensable part to play.

RUSSIAN GEOLOGY IN TRANSLATION

The Geology of the U.S.S.R.

A Short Outline. By D. V. Nalivkin. Translated from the Russian by S. I. Tomkeieff. Translation Editor: J. E. Richey. (International Series of Monographs on Earth Sciences, Vol. 8.) Pp. xii+170+1 map. (London and New York: Pergamon Press, 1960.) 100*s*.

IN recent years new geological maps of the Soviet Union have been published on the scales 1 : 5,000,000 (9 sheets: 1955), 1 : 2,500,000 (19 sheets: 1956), 1 : 7,500,000 (2 sheets: 1958), and 1 : 10,000,000 (1 sheet: 1960). For the first of these the Russian authorities have provided a legend in English. All are magnificent examples of geological cartography, with first-class colouring. The Russian edition of Academician Nalivkin's *Brief Sketch of the Geology of the U.S.S.R.* appeared in 1957, as a paper-back of some 50,000 words written in explanation of the 1 : 5,000,000 sheets. In 1959, an excellent German translation by Dr. E. Schroeder became available (*Fortschritte der sowjetischen Geologie*, Heft 2: Akademie Verlag, Berlin), but unfortunately this was accompanied only by mediocre maps, in black-and-white. The English translation by Prof. S. I. Tomkeieff is issued together with the two 1 : 7,500,000 sheets, specially colour-printed in Moscow for this edition, with place-names in Roman type. It is the first English text on the geology of Russia to be published in book form since the days of Murchison.

The work is, of course, no more than the title implies—a short outline. For each of ten structural divisions of the Soviet Union information is given on topography, stratigraphy, tectonics, magmatism and economic geology, and the range of the book is thus so vast that the coverage is necessarily thin, seldom with more than a few words on any one geological feature. But the observations often suffice to whet the interest, encouraging the specialist reader to search elsewhere for details. The translation is literal, and for the most part it is lucidly written, the few obscurities being due to misplaced punctuation and inadequate proof-reading. As an appendix, a new "Table of Geological Formations in the U.S.S.R." has been added. A special interest resides in the very fine map produced for the British publisher by the U.S.S.R. Ministry of Geology, in an edition of 5,000 copies. The price of the equivalent Cyrillic map (15,000 copies) was, however, only 10½ roubles, and it is unfortunate that the British publication (text with map) should cost five times the Russian original and three times the East German translation.

The publishers state that "this very important volume is the first of its kind to be written in any language". Nevertheless there are other important texts on Russian geology, recently produced overseas and little known to British readers. Among these is a short account (30,000 words) of the *Tectonics of the U.S.S.R.* by the late Academician N. S. Shatsky and Dr. A. A. Bogdanov, initially published in Russian in 1957 as an explanation of a 1 : 5,000,000 tectonic map (9 sheets: 1956). Recently this has been translated into German (*Fortschr. der sowj. Geol.*, Heft 1: 1958), French (Translation No. 1594, Service d'Information Géologique: 1958), and English (*International Geology Review*, No. 1: American Geological Institute, 1959). It provides a particularly readable description of the structural framework of the Soviet Union, drawn on broad lines, and is complementary to Nalivkin's summary.

For detailed information reference can now be made to an extensive Russian work (650,000 words) entitled *The Geological Structure of the U.S.S.R.*, written by more than 150 authors under the direction of Academician A. P. Markovsky and published (1959) in three large volumes. From this text it is possible for the first time to obtain a balanced account of the rock formations of any geological age in any region of the U.S.S.R. The first volume deals with geomorphology and stratigraphy, the second with magmatism and volcanicity, and the third with regional and continental tectonics; and an accompanying portfolio contains 1 : 7,500,000 geological and 1 : 15,000,000 geomorphological maps. The immense task of preparing a Western translation of this highly important monograph has been shouldered by M. Pietresson de Saint Aubin and M. J. Roger of the Centre National de la Recherche Scientifique in Paris, who have already published eight out of a total of 20 fascicules in French, at an average cost of no more than five to six francs per fascicule. This well-produced French work seems likely to remain the most comprehensive Western authority for a good many years.

None of the publications in Russian on the regional geology of the U.S.S.R. meets the present need for a simple, well-illustrated text-book suitable for use, in translation, by Western university students. A noteworthy *Short Course on the Geology of the U.S.S.R.* by Prof. E. Shlygin (Gosgeoltekhizdat, Moscow: 270 pp., 1960) has just appeared, to form a basis for teaching in Russian universities; but this useful catalogue of facts is too unleavened for digestion by British undergraduates. Other Russian texts are similar in character, instructive, but scarcely inspiring. Considering the outstanding advances made by Soviet geologists and the immense range of geological phenomena demonstrable on Russian soil it is surprising that educational books on regional geology are all somewhat turgid and dull.

C. F. DAVIDSON

THE CLOUD OF UNKNOWING

The Gods of Prehistoric Man

By Prof. Johannes Maringer. Translated from the German by Mary Ilford. (The Weidenfeld and Nicolson History of Religion.) Pp. xviii+219+63 photographs. (London: Weidenfeld and Nicolson, 1960.) 42*s*. net.

THIS volume is an English translation of Prof. Maringer's *Vorgeschichtliche Religion*, published in 1956. In accordance with Continental practice, the term 'prehistoric' is used in the absolute sense of world-wide pre-literacy, and thus excludes much of what is still prehistory in English parlance, even if it falls after 2000 B.C. The rendering of the original German text is on the whole adequate, though the translator is clearly unfamiliar with archæological terminology.

Prof. Maringer's avowed purpose is "to define the religious forms of the individual prehistoric periods, to trace the various processes of development, and to arrive at a picture of prehistoric religious manifestations as a whole". This is an exacting task, even for those who regard it as an attainable objective; and there are many prehistorians who will not. But even if one puts this philosophical objection to one side, it must be admitted that the author's treatment of his subject is very uneven in quality. He is clearly most at home with the hunting-magic and the fertility symbols of the Upper Palæolithic era, of which he gives a lucid and succinct account; but by contrast his treatment of later periods is superficial, and in places positively archaic. The whole problem of the symbolic iconography of the passage-graves of Atlantic Europe is scarcely discussed at all; and it is not encouraging to be told that Stonehenge consists of three stone circles, and is orientated to the sunrise at the mid-winter solstice.

The principal defect in this book, and indeed of the attitude to prehistory which it represents, is a failure to admit and to understand the inescapable limitations of archæological evidence. We can re-create the past meaningfully, from its surviving material remains, only to the extent that the physical results of human behaviour can be translated back into the states of mind of which they are the expression. In matters of pre-literate technology and economics, this process of translation is relatively easy and reliable. In the field of social and political institutions it becomes increasingly difficult; and for religion it is virtually impossible. Religion, in the last analysis, belongs to the world of ideas and emotions, and finds its physical expression only in ritual. But, in the absence of the written or verbal explanations available to the historian and the ethnographer, ritual cannot reliably be translated back into terms of belief, simply because there is no one-to-one correspondence between concept and action.

This difficulty is tacitly, though never consciously, recognized by the author in his frequent citations of modern ethnographic parallels for the phenomena of prehistory. But analogies of this kind serve only to enlarge the field of conjecture. They cannot be used to explain the rituals of prehistory, unless it is assumed that human behaviour has survived unchanged, as a kind of museum specimen, from the prehistoric past into the present. If there is one thing that archæology can demonstrate, it is the total falsity of this assumption.

R. J. C. Atkinson

AGRICULTURAL AVIATION

Report of the First International Agricultural Aviation Conference

September 15–18, 1959, Cranfield, England. Pp. 429. (The Hague: International Agricultural Aviation Centre, 1960.) 50*s*.; 7 dollars; 29 D.M.

Agricultural aviation is not yet so widely accepted in Europe as it is in the U.S.A., for example, mainly because in Europe the need is smaller and some of the difficulties are greater. Many contributors to this report come from Europe and are accordingly considering the value of work done from the air in high-farming areas.

N. D. Norman in a paper reviewing the economics of aerial contract work on farms, shows that costs are high and that earning power is limited by a short working season, by limited periods of flying weather and by the working-rate of the aeroplane on its main tasks of applying chemicals to crops. He shows how it is difficult to operate such a contract service profitably in England.

More information is required about each of several potential ways of raising income above expenditure before they can be offered to the industry. It is difficult, in this sphere, to predict that a change will cause an improvement, especially in countries of relatively small fields, dense populations and many hazards both to flying and of applying chemicals, some of which may not contaminate down-wind crops.

These and other matters of safety, legislation, operating techniques, equipment and aircraft, in fact almost the whole art of agricultural aviation, are touched on in the report, and the treatments are necessarily brief and qualitative. However, such subjects which form the basis of the industry might have been presented with more quantitative information than, with exceptions, the papers contain.

Thus, although the agronomic basis of aerial agriculture is surveyed by R. C. Amsden, the individual papers to support him provide only a little evidence to show how and where an aeroplane is either as effective as, or more profitable than, a ground sprayer. R. A. Powell shows that fertilizer distribution from an aeroplane can be as regular as that from most distributors on the ground. No other paper provides critical evidence to demonstrate what advantages an aeroplane has over a ground machine. Wheel damage, onset of disease in the wheel tracks, the value of timely application of pesticides and of fertilizers are mentioned, but without such data as would convince a farmer.

The dangers of low-level flying and of applying poisonous chemicals are considerable. D. Yeo reviews the dynamics of small particles in the air-flow near an aeroplane close to the crop and shows that although contamination of down-wind crops can be serious, precautions to prevent it can be successful. E. F. Edson and others show that no harm from toxic chemicals has been detected, except when accidents have occurred, but point out that the pilot has to take great care in order to prevent pollution completely.

In a countryside full of obstacles a pilot has to take great care of himself also. It is therefore questionable whether F. E. Weick's paper is as relevant for France as it may be for Florida. He quotes, without supporting evidence, 60–100 m.p.h. as a suitable flying speed. But Latimer–Needham's graphs in the economics section show that, at this speed, 90 per cent of flying time is consumed in half-mile long turns at the end of the field. Possibly, therefore, a lower flying speed is desirable in order to work more quickly, to be safe and also in order to fly close to the ground to avoid pesticide pollution. It is possible also that a monoplane has to give way to a rotary-wing aircraft as suggested in de Ree's paper.

The aircraft used at present are light monoplanes and war-surplus biplanes. Sir Miles Thomas points out how much better the helicopter is for the work. However, the helicopters used so far have been expensive and less reliable than fixed wing aeroplanes. The Kolibrie helicopter, as described by de Ree, seems an improvement in both respects despite its high fuel consumption. Surprisingly, the autogyro, which appears to have the merit of a helicopter for this work at the price of a light plane, receives no more than a mention along with ducted fans and other unconventional, vertical take-off aircraft.

European conditions may be sufficiently different from those in the United States and Australia for changes in aircraft, application techniques and organization of the work to be necessary, just as fertilizer and plant protection programmes differ. This report mainly poses the questions to which answers are needed to help the industry to prosper, and it is valuable for this alone. It is well produced, but an index would be helpful.

R. J. Courshee

THEORY OF BANACH ALGEBRAS

General Theory of Banach Algebras

By Prof. Charles E. Rickart. (The University Series in Higher Mathematics.) Pp. xi+394. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 79*s*.

THIS book gives a comprehensive and detailed account of the theory of Banach algebras, and should be of great value to research workers in this field. The subject is a very modern one, the foundations for a general theory being laid by Gelfand in a paper published in 1941, but announced in 1939 (Normierte Ringe, *Recueil Math.* (*Mat. Sbornik*), N.S., 9, 3; 1941).

The contents of the book are as follows. Chapter 1, "Fundamentals"; this includes definitions, the regular representations, complexification of a real normed algebra, the groups of regular and quasi-regular elements, topological divisors of zero, the spectrum, and normed division algebras. Chapter 2, "The Radical, Semi-simplicity and the Structure Spaces"; this includes ideals and difference algebras, representations, the radical, primitive Banach algebras, uniqueness of the norm topology and the fundamental isomorphism theorem, structure of semi-simple Banach algebras, the structure spaces, completely regular algebras and annihilator algebras. Chapter 3, "Commutative Banach Algebras"; this includes the carrier space and the Gelfand representation theorem, algebras of functions, the Šilov boundary, representations of the carrier space, homomorphisms of certain function algebras into a Banach algebra, direct-sum decompositions and related results and completely regular commutative Banach algebras. Chapter 4, "Algebras with an Involution"; this includes miscellaneous properties of *-algebras, commutative *-algebras, self-dual vector spaces and *-representations, representations on Hilbert space, positive functionals and irreducible *-representations, symmetric *-algebras, general properties of B*-algebras, structure of ideals and representations of B*-algebras, and Banach*-algebras with minimal ideals.

There is, finally, a lengthy appendix on examples and applications, under three headings: (*a*) "Algebras of Operators"; (*b*) "Algebras of Functions"; (*c*) "Group Algebras". Included in (*a*) are the algebras $\mathfrak{B}(\mathfrak{X})$ and $\mathfrak{B}(\mathfrak{H})$, the algebra $\mathfrak{I}\mathfrak{C}$ of compact operators, the Schmidt-class $\mathfrak{I}\mathfrak{S}$, the trace-class $\mathfrak{I}\mathfrak{T}$, W^* and AW^* algebras, and spectral operators; under (*b*) are included the algebra $C(\Omega)$ for certain special Ω, the l^p-algebras, functions with absolutely convergent Fourier series, functions of class $C^{(n)}$, continuous functions of bounded variation, holomorphic functions of one variable, holomorphic functions of several variables, a non-self-adjoint algebra with Šilov boundary equal to the carrier space, non-existence of the Šilov boundary, algebras of set functions on the line, some radical algebras, and algebras of power series; while (*c*) includes the algebra $L^1(\mathfrak{G})$, locally compact abelian groups, group algebras of a compact group, almost periodic functions on groups, and group algebras of operators.

The book is evidently written for mature mathematicians who already have at least a slight acquaintance with the subject. Those not so equipped would, apart from the difficulty of the text, find the lack of simple illustrative examples in the four chapters a serious stumbling-block. The appendix would not supply this need, as the applications are given in a very condensed manner, requiring a high degree of maturity from anyone hoping to profit from it. However, for professional mathematicians having already some knowledge of the subject, the book will undoubtedly be of great value.

R. G. Cooke

DR. ERIC C. S. MEGAW

A Backroom Boy

By Arthur Stanley. Pp. vi+106. (Belfast: W. Erskine Mayne, Ltd., 1960.) 10*s*. 6*d*. net.

THIS little book, written by a father as a memorial to his brilliant son, describes the life of Dr. Eric C. S. Megaw, who was director of physical research at the Admiralty when he died at the early age of forty-eight years.

As a schoolboy Megaw acquired a sound knowledge of the French language, including facility in speaking it. Also, an interest in mechanical things led him at the age of fourteen to a keen interest in amateur radio experiments. This was encouraged by a short period in the Signals Section of the Officers' Training Corps, and in 1924 he was reputed to be the first amateur to send radio signals from Ireland. In the following year he established the first radio contact between Ireland and Australia.

The book outlines in the author's 'domestic' style Megaw's scientific career which was described in the obituary notices published in *Nature* (177, 409; 1956). The factual account is supplemented by extracts from his personal letters, which illustrate his acute power of observation and close attention to the work in hand. The final chapters of the book comprise extracts from letters received by Mrs. Megaw from officials and colleagues who were closely associated with, and very appreciative of, the scientific work of her late husband.

This volume is prefaced by a letter from Sir Eric Ashby, formerly vice-chancellor of the Queen's University of Belfast, where Megaw obtained both his bachelor's and doctoral degree in science. Sir Eric welcomes this memorial as a reassurance to both teachers and students, in describing "how the most satisfying career can be built on quiet, steady work, without publicity, without ostentation, and without neglecting family life, or music, or literature".

A brief appendix lists Megaw's published papers, including that with two of his colleagues, which was published posthumously and was awarded the highest premium of the Institution of Electrical Engineers.

R. L. Smith-Rose

Structure and Properties of Thin Films

Proceedings of an International Conference held at Bolton Landing, New York, September 9-11, 1959. Edited by A. Neugebauer, J. B. Newkirk and D. A. Vermilyea. (Sponsored by Air Force Office of Scientific Research, Air Research and Development Command, and the General Electric Research Laboratory.) Pp. xiv+561. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 120*s*. net.

THERE are so many scientific conferences organized nowadays that one wonders how anyone has time to carry out the research which is discussed at such meetings. It is inevitable that many scientists who would like to participate in a particular conference are prevented from doing so; for these people a well-produced book of Proceedings can be invaluable. Even the participants can profit from a more leisurely perusal of the flood of new ideas. The present report is an excellent example of its kind. It was published within a few months of the conference and is extremely well produced, both typographically and editorially.

The papers are divided into six sections dealing with the formation of thin films, with their mechanical, electrical and magnetic properties, with chemical interactions at the surface and the general theory of surfaces. A regrettable omission is a section on the optical properties of thin films, although optical techniques figure occasionally in the individual papers. It would be invidious to select any particular report from so wide a field; many of the papers have a distinct technological bias and the research described is aimed chiefly at a particular application, for example, computer devices. It would have been better if the title had been restricted to thin metal films—the papers deal almost exclusively with the properties of such interesting substances as aluminium, copper, gold, iron, nickel, silver, etc.

The editors are to be congratulated on the high standard of the illustrations and on the provision of both an author and a subject index. The discussions of the papers are fully reported and in many cases are quite as stimulating as the original report.

J. RING

An Introduction to Differential Geometry

By T. J. Willmore. Pp. x+317. (Oxford: Clarendon Press; London: Oxford University Press, 1959.) 35*s*. net.

THE elegant domain of classical differential geometry in three dimensions is now somewhat out-moded. But it retains a practical value, for example, in design problems, and forms a good, perhaps essential, prelude to work in n dimensions. Dr. Willmore's book deals with both aspects. In the first part, it gives the main results about curvature and torsion of curves and curvature of surfaces, using vector notation; this is very clear, though some use of kinematical concepts might have rendered it more vivid. There is also an excellent introduction to differential geometry in the large, a topic of increasing importance not generously treated in English texts. In the second section, the space is n-space, and tensor algebra and tensor calculus are developed *ab initio* to cope with Riemannian geometry and with the generalizations of the results in Part I. There is a good supply of exercises, mainly from University of Liverpool examinations, and plenty of bibliographical references for further reading. The first part of the book must be counted as essential to any honours mathematics student, while the second part forms a valuable first text for those who intend to begin research in global theory and differentiable varieties.

General Degree Applied Mathematics

By S. L. Green and J. E. C. Gliddon. Pp. iv+346. (London: University Tutorial Press, Ltd., 1959.) 18*s*.

THIS text, with Mr. Green's *Dynamics*, covers the applied mathematics (excluding statistics) required by the London B.Sc. (General), revised regulations. The authors have produced just the book for the average undergraduate, who will not complain, may even rejoice, at the limitation in outlook values imposed by a careful observance of the syllabus. The book deals with virtual work, statics of beams and strings, normal modes, vibrations of strings, hydrodynamics, gravitation and electrostatics. Vectors are used freely but sensibly, though it is sad to see that they cannot yet be taken for granted at this level, and require sixty pages of treatment. The bookwork is presented in clear and easy style; in some places, as, for example, in the vector integral theorems, geometrical intuition is used freely to supplement or avoid analytical rigour. Every point is driven home by worked examples, carefully selected from recent examination papers, while the British tradition of plenty of exercises for the reader is fully respected. It is hard to think of a better book for the particular purpose; and if the purpose seems not always sufficiently up to date, that is no fault of the authors The revised syllabus itself may soon demand revision.

Nutrition and Physical Fitness

By Dr. L. Jean Bogert. Seventh edition. Pp. xi+613. (Philadelphia and London: W. B. Saunders Company, 1960.) 6 dollars; 42*s*.

THE earlier editions of Dr. Bogert's book had the laudable aim of presenting a comprehensive view of the whole subject of nutrition in fairly simple language suitable for students who had no previous knowledge of chemistry. As it is more or less impossible to discuss many facets of nutritional science without some reference to their chemical connexions, the present edition has been improved by inclusion of some simplified material on the chemical constitution of carbohydrates, fats and proteins. Some graphically illustrated ideas on the digestion and absorption of nutrients and on the functions of vitamins and enzymes are included. The first two parts of the book on body-needs and body-functions have been considerably rewritten, and many illustrations and diagrams have been added. Lists of references for supplementary reading have been extended, which increases the usefulness of the text. Within the limitations necessarily imposed by the need to simplify statements and to be dogmatic on points which might otherwise be obscure for the students for whom the book is intended, *Nutrition and Physical Fitness* is a good teaching text-book for introductory courses in nutrition. Its ideas on meal planning are designed for the American way of life, but they can be adapted for other countries. Some notes on food fallacies and their refutation might well be studied by all who have concern with knowledge of foods and nutrition.

RELATIVE EFFICIENCIES OF INDIAN LANGUAGES

By Dr. B. S. RAMAKRISHNA, K. K. NAIR, V. N. CHIPLUNKAR, B. S. ATAL,
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THE concept of information and its measure developed in telecommunication engineering during the past decade or so have made it possible to investigate certain questions in linguistics in a quantitative manner. We describe here briefly a method of comparing the relative efficiencies of different languages and their scripts as means of communication. The efficiencies of some of the modern Indian languages, namely, Hindi, Marathi, Tamil, Malayalam, Telugu and Kannada, have been evaluated with respect to English, which was chosen as a common reference. The concepts on which the comparisons depend, however, are of general applicability and are by no means restricted to Indian languages alone. We shall, indeed, introduce these notions here by referring to a similar comparison between English and German which we made prior to the study of the Indian languages.

The key-note of this investigation is that where different languages can be employed to serve the same end-purpose, we can compare their relative performances as alternative means of communication using the tools of information theory. This work is based on two notions: the first of these is that, if we accept that language is a code for the purpose of communication, linguistic statements which convey the same meaning in different languages can be considered as alternative forms of encoding the same semantic content into linguistic symbols. The semantic equivalence of messages in different languages, however, does not imply that their informational entropies are also equal. (The informational entropy associated with a symbol such as a letter, phoneme, etc., of a message is defined in communication theory, in a manner analogous to the thermodynamical entropy, as $-\Sigma_i p_i \log p_i$, where the p_i's represent the probabilities of occurrence of the symbols obtained on a statistical basis from sufficiently long messages. The total entropy of a message containing N symbols is therefore $-N\,\Sigma_i\, p_i \log p_i$. When the logarithms are taken to the base 2, the unit of informational entropy is called a bit or, sometimes, a selective bit, to emphasize the selective role of information. The definition of entropy can be extended to include the statistical constraints between symbols, but we shall use here only the entropy based on letter probabilities, as all our comparisons are based on letter frequencies only. For a more detailed discussion of the concept of entropy see, for example, ref. 1.) It is well known that the entropy of a message is the proper measure of the cost of its transmission, storage, etc. It follows, therefore, that the entropies of semantically equivalent statements in different languages constitute inverse measures of the relative efficiencies with which the languages encode semantic content into linguistic symbols.

To obtain semantically equivalent statements in two languages one must take suitable samples of texts in one of the two languages and their translations in the other. Thus in a comparison of English and German[2] (two closely related languages) it was found that when the original text was in English and the translation in German, 171,017 bits of information in English are equivalent to 207,590 bits in German, which seems to imply that one bit in English has about the same semantic value as 1·21 bits in German. On the other hand, with the original in German and the translation in English, 163,984 bits in English prove to be semantically equivalent to 175,491 bits in German, leading now to an exchange ratio of 1·07 bits of German for one bit of English. From similar comparisons of English and different Indian languages, it appears that the exchange ratio is always relatively more favourable to a language when the original is taken in that language.

This situation can be explained on the following information theoretical model of translation, namely, that translation is a process of the transformation of the code in which we aim at leaving the semantic content invariant despite a certain inherent tendency for equivocation or semantic ambiguity arising in the translated text, which, in the terminology of the communication theory, may be called semantic noise. To preserve the semantic content, therefore, the translator must overcome the noise by using additional bits of information. Thus each bit has to be paid for—not only its proper equivalent in the language of translation but also the additional bits required to overcome the noise. If we assume that x bits in German are the proper semantic equivalent of one bit in English and that y additional bits are needed to overcome the noise associated with the translation of each bit in both directions, we obtain the pair of simultaneous equations:

$$171{,}017\ (x + y) = 207{,}590$$
$$175{,}491\ (x^{-1} + y) = 163{,}984$$

for the English to German and German to English translations respectively, which yield $x = 1{\cdot}15$ and $y = 0{\cdot}065$. This makes English relatively more efficient in that it requires about 15 per cent fewer bits for transmitting the same semantic content than does German. Similar comparisons established the relations between English and the different Indian languages as shown in Table 1.

Table 1. SEMANTIC EFFICIENCIES OF INDIAN LANGUAGES RELATIVE TO ENGLISH

Indian language	Number of bits semantically equivalent to one bit in English x	Additional bits required for each bit translated y
Hindi	1·34	0·22
Tamil	1·09	0·17
Malayalam	1·24	0·08
Telugu	1·14	0·04
Kannada	1·14	0·07

Now what makes a language more or less efficient than another for communicating semantic content ? Evidently a language becomes less efficient either if ideas are expressed in the language in a redundant form or if it uses relatively longer words to express the same notions. It is possible that the relatively high semantic efficiency we have noted above for English could be due to the large number of short words in that language. (It may be noted that the average length of a word in English is about 4·5 letters, whereas it is about 6 letters in German.) Further analysis is needed before we can partition the inefficiency of a language into components attributable to these two causes.

The other notion introduced here relates to the comparison of the verbal-to-orthographic transcription schemes of different languages. Just as speech is a code for expressing our thoughts, so do the different alphabetic and syllabic forms of writing constitute codes for transcribing the sounds of speech into written symbols. Whenever, therefore, it is possible to transcribe a given phonetic pattern by two or more scripts, we may regard the corresponding written symbols as alternative codes the phonetic content of which remains the same. We have thus a basis for comparing different scripts, once we have chosen a criterion of merit.

One of the chief merits of a script is the speed with which it can be written. If we digress for a short while to examine the history of writing, we see how the evolution of different scripts was greatly influenced by the speed with which their characters could be 'written' on the materials available during different periods. The transition from pictographic writing to the use of word-signs, then the syllabary, and finally the alphabet in Mesopotamia, and likewise from the hieroglyphic writing to the hieratic and demotic scripts in Egypt, have all been steps towards achieving faster speed. Speed, however, has never been the sole determinant. It would be more appropriate to say that the evolution of writing follows a compromise between the opposing needs of speed on one hand and legibility on the other. Indeed, the progress of writing seems to be governed by principles similar to those existing in human speech. For example, it will be recalled that some years ago Zipf[3] had observed that if the words in a language are ranked in the order of their frequency of occurrence then the product of the rank and the frequency of each word is nearly a constant. Information theorists[4] have tried to explain this relationship on the basis that it costs a certain effort to speak each word, and that this cost may be taken proportional to the length of the word. It is then shown that Zipf's relation between the rank and frequency is precisely the one which makes the average cost per word a minimum in accordance with Zipf's Principle of Least Effort. A parallel case is to be found in the syllabic scheme of writing used in the Indian languages : the vowel sounds which are more frequent than the consonantal sounds are not written separately when they follow a consonant, but merely modify the consonantal symbols with which they are associated, thus minimizing the effort needed in writing.

Zipf has also directed attention to how certain words acquire multiple meanings in a language as a result of two opposing forces, namely, the economy of the speaker, who favours the fewest possible number of words to refer to whatever he means (just one, if feasible, according to Zipf) and the needs of the listener, who favours a separate word for each 'referent' which the speaker intended to refer. In each language a balance suited to the needs of its community is achieved between these opposing requirements by certain words acquiring multiple meanings, thus limiting the size of its vocabulary. An analogous principle also seems to underlie the evolution of writing at various stages of its development. When picture writing was found to be inadequate as a means of unambiguous communication to the growing needs of settled communities, the early scribes had to increase the detail in the picture as well as their number. The device of using the same picture sign to represent homonyms (that is, words having the same sound value but different meanings) which was then developed helped the scribe to achieve some economy in writing. A further step in this direction was the juxtaposition of the symbols corresponding to the different syllables in a word whenever this was possible. This process reduced even further the number of different signs required for communication because the total number of different syllables required is generally less than the number of words. Thus, we are told (see, for example, the article on "Recording and Writing", by S. H. Hooke, in *A History of Technology*, 1, Oxford Univ. Press, 1954), that while there were about 2,000 word signs in use in Mesopotamia about 3600 B.C., by the end of the fourth millennium the syllabary consisted of only about 600 signs. The principle of alphabetic writing in which the symbols came to represent the individual speech sounds was the last step which further reduced the number of different symbols needed for writing. On the other hand, in the development of the miniscule or the lower-case letters from the majescule or upper-case letters, we have an instance in which legibility rather than the desire for speed played the main part. As the capital letters were written in haste, the characters suffered in legibility, and to preserve the distinction among the letters some of them were given ascending strokes as in *k* and *h* and others descending strokes as in *p*, *q*, etc.

Whatever may be the factors influencing the evolution of writing, we shall accept here the Roman and Indian scripts in the form in which they are handed down to us and confine ourselves to the question whether the alphabetic form of writing of the Roman script has any advantage over the syllabic form of writing of the Indian languages in the matter of speed. To answer this question, writing was treated as an acquired skill, and a subjective test minimizing the effect of personal factors was designed as follows : from the phonemes common to English and the Indian languages (which account for more than 80 per cent of their occurrences in any of these languages) a set of 100 nonsense syllables were composed and were then transcribed by the Roman script and the several Indian scripts on separate test sheets. A number of subjects (about 60) who had had sufficient (but not necessarily equal) practice in writing the Roman script and one of the Indian scripts were asked to copy these syllables on the same sheets and in the same scripts. Thus a subject who could write Roman script and Hindi script (Devanagari) wrote the same 100 sounds in Roman and Devanagari scripts when he completed the test sheets. The times taken by the different individuals in the two scripts were noted. This procedure was followed with different types of syllables such as *CV* (consonant vowel), *VC*, *CVC*, *CCV*, etc., and the several Indian languages, using each time Roman script as a common reference. For each type of syllable, the subjects furnish a bivariate sample of data; namely, the times required for transcribing the same phonetic content by the Roman script and one of the Indian scripts.

Since the observed times are likely to be influenced by the extent of practice of the individuals in the two scripts, the subjects were classified on the basis of their practice in writing the two scripts into three categories, namely, those that have relatively more practice in writing the Roman script, those that have relatively more practice in writing the Indian script, and those that have about equal practice in both scripts. The observed writing times will not only be influenced by the scripts themselves but also by the writing speed and practice of the individuals in them, as well as random errors. Considerable attention was therefore given to investigating the significance of the effect of practice on the observed writing times. Study of the cross-correlation between the writing times of the individuals in the two scripts showed that, in general, a person who is a fast (or slow) writer in one script also tends to be a fast (or slow) writer in the other script, irrespective of the extent of his practice in the two scripts (provided he has had practice beyond a certain minimum).

Following now our assertion that the different scripts are but different modes of transcription, we can now classify the mean writing times of the different categories with the script and the practice as the two variables of classification[5]. Table 2 shows a typical partition of the mean writing times ξ_{ij} of the different categories in the Roman and an Indian script (Telegu) into components which are attributable to the effects of (1) script, (2) practice and (3) random errors measured from the grand mean $\mu = \Sigma_{ij}\,\xi_{ij}/6$. The cell means ξ_{ij} $(i = 1, 2, 3;\ j = 1, 2)$ differ from each other not only because of population variance and random errors, but also because of intrinsic differences in the scripts and differences in the practice of the different categories.

We assume that any cell mean ξ_{ij} can be written as:

$$\xi_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where μ is the same for all the cells, α_i $(i = 1, 2, 3)$ is a constant for all cells in the i-th row, β_j $(j = 1, 2)$ is the same for all the cells in the j-th column and ε_{ij} a random variable which varies from cell to cell and has a sampling distribution with mean zero. (This assumption implies that there is no interaction between the row effects and column effects.) The quantities α, β and ε are determined so that

$$\sum_{i=1}^{3}\alpha_i = 0,\quad \sum_{j=1}^{2}\beta_i = 0,\quad \sum_{i=1}^{3}\varepsilon_{ij} = \sum_{j=1}^{2}\varepsilon_{ij} = 0 \qquad (i = 1, 2, 3;\ j = 1, 2)$$

Table 2. CLASSIFICATION OF THE MEAN TRANSCRIPTION TIMES FOR THE *CV* TYPE OF SYLLABLES WITH SCRIPT AND PRACTICE AS THE TWO VARIABLES AND THE PARTITION OF THESE MEANS INTO COMPONENTS DUE TO THE EFFECTS OF SCRIPT, PRACTICE AND RANDOM ERRORS

Category of practice	Mean transcription (on writing) times of *CV* type syllables	
	In Roman script (sec.)	In Telugu script (sec.)
English practised category	$\xi_{11} = \mu + \alpha_1 + \beta_1 + \varepsilon_{11}$ $176 = 164 + 7 + 13 - 8$	$\xi_{12} = \mu + \alpha_1 + \beta_2 + \varepsilon_{12}$ $166 = 164 + 7 - 13 + 8$
Telugu practised category	$\xi_{21} = \mu + \alpha_2 + \beta_1 + \varepsilon_{21}$ $192 = 164 + 7{\cdot}5 + 13 + 7{\cdot}5$	$\xi_{22} = \mu + \alpha_2 + \beta_2 + \varepsilon_{22}$ $151 = 164 + 7{\cdot}5 - 13 - 7{\cdot}5$
Equal practice category	$\xi_{31} = \mu + \alpha_3 + \beta_1 + \varepsilon_{31}$ $163 = 164 - 14{\cdot}5 + 13 + 0{\cdot}5$	$\xi_{32} = \mu + \alpha_3 + \beta_2 + \varepsilon_{32}$ $136 = 164 - 14{\cdot}5 - 13 - 0{\cdot}5$

In our case, the quantity μ establishes a common reference of the mean writing time for the different scripts and the practice groups, and serves as the balance point about which the practice effects represented by the α's and the script effects represented by the β's produce deviations. The row-variables measure the effects of practice of the different categories from this common reference and are thus independent of the particular script. A positive value of α for a particular practice-category implies that its members are on the average slow writers, whatever the script. Similarly the column-variables β's measure the effects of the scripts independently of the practice of the different categories. A positive value of β for a script implies that it is a more time-consuming one than the other. If there were no differences in practice among the three categories, $\alpha_1 = \alpha_2 = \alpha_3 = 0$ and the mean writing times in the Roman and the Indian (Telugu) scripts would have been $\mu + \beta_1$ and $\mu + \beta_2$, apart from the effect of random variations represented by the quantities ε_{ij}, which we may expect to tend to zero as the sizes of the samples are increased. The quantities $\mu + \beta_1$ and $\mu + \beta_2$ are therefore the proper measures of the times required for transcribing the same phonetic content by the Roman and Telugu scripts, free from the differences in practice of the different categories The numerical values of the quantities ξ_{ij} and their components μ, α's, β's, etc., are given immediately below them in each cell for the *CV* type of syllables We can now arrive at an estimate of the relative time requirements of the two scripts by similarly analysing the data for the other types of syllables and taking the relative frequencies of the different types of syllables in the Indian languages into account.

The final results show that while in the case of *CV* and *CCV* type of syllables most of the scripts of the Indian languages studied here have a slight advantage (requiring about 10 per cent less time than the Roman script) this is not the case in respect of the *V*, *VC* and *CVC* type of syllables. This might have been anticipated, as the syllabary enables the entire *CV* or *CCV* type of syllable to be represented by a single character with relatively minor modifications. It is also evident that even in the cases where the Indian scripts save in time, this saving is partly or even completely off-set by the fact that a cursive hand is not possible with them to the same extent as with the Roman script. It appears that the adaptation of the Roman script to Indian languages results in a reduction of about 5 per cent in the writing time for Tamil, Kannada and Hindi, and an increase of about the same magnitude in the case of Telugu and Malayalam.

While the numerical results given here are subject to the limitations inherent in the statistical sampling that is necessary, we should like to focus attention on the possibility of investigating such questions in a quantitative manner. It is proposed to publish the details of these investigations in the form of a monograph.

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BRITISH NORMAL THRESHOLD OF HEARING

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IN 1954 a British Standard[1] on the normal threshold of hearing for pure tones by earphone listening was published. The values specified in the standard are based on two investigations[2,3] carried out in the United Kingdom in which tests were made on more than 1,200 ears of otologically normal subjects of ages 18–25 years.

A subsequent investigation by Knight and Coles[4] has added further weight to the reliability of the Standard, the values for 74 male subjects agreeing within 1·4 db. at frequencies from 250 to 4,000 c./s. inclusive. Further experimental confirmation has been given by Hinchcliffe[5].

In the British Standard the threshold is expressed in two ways: (*a*) As the minimum audible pressure measured at the entrance to the external auditory meatus, when the sound pressure is produced by an earphone applied to the ear. (*b*) In terms of the sound pressure set up in an artificial ear complying with B.S.2042 (ref. 6) by an earphone of a specific pattern when actuated by the voltage corresponding to the normal threshold referred to in (*a*).

We are here concerned only with the latter, termed the 'equivalent normal threshold sound pressure' (ENTSP).

The ENTSP is dependent on the pattern of earphone, and for the British Standard this is the type 4026*A* manufactured by Standard Telephones and Cables, Ltd., London. If the ENTSP for earphones of other types is required, this is obtainable by means of loudness-balancing against a standard earphone of the reference pattern, and for this purpose the British Standard[1] recommends the employment of at least twenty observers. While loudness balancing must remain, for the time being, the primary method of calibrating earphones for audiometric use when the type involved is other than the appropriate national reference pattern, the accumulation of test results now makes it possible to present, at least provisionally, some values of the ENTSP corresponding to the British Standard threshold of hearing for a few of the other earphone patterns in common use.

The results of such comparisons carried out over the past few years at the National Physical Laboratory are presented in Table 1. Meanwhile, an investigation has also been carried out to assess the reliability of these subjective transfers[7].

By measurements of the objective sensitivities of earphones of these types on the B.S. artificial ear and 9*A* coupler, respectively, we are able also to express the British Standard threshold of hearing in terms of sound pressure in the 9*A* coupler (columns headed '9*A*'). The information is then in a form in which direct comparison can be made with results on earphones and audiometers calibrated to other threshold standards, principally American (for example, those given in refs. 8 and 9), which are based on the 9*A* coupler.

It is well known that a considerable divergence exists between the current British and American

Table 1. EQUIVALENT NORMAL THRESHOLD SOUND PRESSURES
(db. re 1 dyne/cm.^{2})

Frequency (c./s.)	4026*A*			705*A*			*TDH*39(*MX*41/*AR*)*				*PDR*1(*ADC*)*			*PDR*10 (*MX*41/*AR*)*			*DT*48†	
	B.S.	9*A*	Derived 9*A*	B.S.	9*A*	Derived 9*A*	B.S.	9*A*	Derived 9*A* (*a*)	Derived 9*A* (*b*)	B.S.	9*A*	Derived 9*A*	B.S.	9*A*	Derived 9*A*	B.S.	9*A*
80	−13·5	−7·0	—	—	—	—	−8·5	−5·0	—	—	—	—	—	—	—	—	−13·5	−9·5
125	−29·5	−25·0	−22·5	−30·5	−29·0	−28·5	−25·0	−23·5	—	−31·5	−27·5	−26·5	−29·5	−25·0	−23·0	−28·0	−31·5	−29·0
250	−44·5	−42·0	−41·5	−47·5	−47·5	−46·0	−43·0	−43·5	−45·0	−46·0	−44·0	−44·5	−45·5	−42·5	−42·5	−43·5	−45·0	−44·5
500	−62·0	−60·0	−60·0	−60·5	−60·0	−59·5	−59·0	−59·0	−59·5	−60·0	−60·5	−61·0	−59·5	−60·5	−60·5	−60·0	−61·0	−59·5
1,000	−68·0	−67·0	−66·0	−65·0	−65·0	−65·0	−63·0	−64·0	−65·5	−64·5	−65·0	−65·5	−67·0	−64·0	−64·5	−66·0	−66·5	−66·0
1,500	−66·5	−66·0	−66·0	−64·0	−64·0	−65·0	−62·5	−64·0	—	—	−64·0	−65·5	—	−63·0	−63·0	—	−65·5	−65·0
2,000	−65·0	−66·0	−66·5	−63·0	−64·5	−64·5	−62·5	−65·0	−65·0	−63·5	−63·0	−66·5	−64·5	−64·0	−66·5	−66·0	−64·5	−66·5
3,000	−68·0	−67·5	−67·0	−64·0	−65·0	−66·0	−64·0	−64·0	−61·0	—	−64·0	−66·0	—	−63·5	−65·0	—	−68·5	−70·0
4,000	−65·0	−64·0	−62·5	−61·5	−63·5	−66·5	−62·5	−64·0	−67·0	−67·0	−61·0	−61·0	−60·5	−64·0	−64·0	−67·5	−68·0	−69·0
6,000	−65·0	−59·5	−59·5	−70·0	−66·5	−63·0	−56·5	−51·0	−51·0	—	−62·5	−63·5	—	−58·0	−56·0	—	−70·0	−66·0
8,000	−65·0	−54·5	−53·5	−77·5	−64·5	−64·0	−67·5	−58·5	−59·0	−58·0	−69·5	−61·0	−60·0	−63·5	−53·0	−61·5	−71·0	−59·5
10,000	−58·5	−54·0	−56·0	−67·0	−60·0	−49·5	−57·0	−47·0	—	—	−59·0	−53·5	—	−61·5	−57·0	—	−50·5	−42·5

* *MX*41/*AR* and *ADC* are descriptions of the earcaps.

† The soft rubber earcap supplied with this earphone was removed for measurement on the 9*A* coupler only, in accordance with German practice.

(*a*) From ref. 11, (*b*), from ref. 12.

Table 2. ESTIMATE OF THE INTRINSIC DIFFERENCE BETWEEN BRITISH STANDARD THRESHOLD OF HEARING AND AMERICAN VALUES

f (c./s.)	British threshold S.P.L. —A.S.A. threshold S.P.L.	British threshold S.P.L. —threshold S.P.L. given by Albrite *et al.* (ref. 10)
125	− 9	+ 1·5
250	− 11·5	+ 2·5
500	− 10	+ 1·5
1,000	− 8	+ 1·5
1,500	—	+ 0·5
2,000	− 7·5	− 1·0
3,000	—	− 5·0
4,000	− 7·5	− 2·0
6,000	—	− 5·0
8,000	− 11	− 5·0
10,000	—	− 3·0

standards[1,8]. However, recent American work[10] has shown that when thresholds are measured under similar experimental conditions and when the same method of selection of subjects is used, the discrepancy between the threshold of hearing measured in the United States and in Britain to a large extent disappears. At the present time we are able to make the provisional estimate of the residual difference shown in Table 2, as a result of interlaboratory exchanges of the actual earphones employed, together with some indirect subjective transfers discussed below. This should not be regarded as final, however, as the resolution of this discrepancy is at present under consideration by Technical Committee 43 of the International Standards Organization.

To illustrate the comparison between the new American data[10] and the British, we have derived from American data six estimates of the British threshold referred to various earphones, and these are shown for comparison with the British data under the columns 'derived 9*A*' in Table 1. The values have been obtained as follows: To the equivalent threshold sound pressures for the various earphones (given in refs. 8, 9 and 11 in terms of the current American Standard) we have added a correction (Cook, R. K., private communication) to bring them into line with the new data of Albrite *et al.* In the case of the *TDH*39, values already expressed in terms of Albrite's threshold were available as a result of subjective comparisons between the *TDH*39, 4026*A* and 705*A* types carried out by A. Glorig (private communication). To the corrected values we have added a further adjustment for the estimated difference between the Walter Reed and British threshold determinations, shown in Table 2.

The resulting values are the 'derived 9*A*' ENTSP. The extent to which these depart from the values shown in the corresponding columns headed '9*A*' is a measure of the combined subjective errors involved in transfers between the reference earphones and those of the other types shown, objective errors (which are probably small), and the failure of the particular earphones used in the various comparisons to conform exactly to pattern (that is, the ratio of subjective sensitivities may not be precisely equal to the ratio of sensitivities on the artificial ears). The departures are in most cases quite small and non-systematic (mean 1·2 db., maximum 3·5 db.) over the range 250–6,000 c./s., which is regarded as satisfactory in view of the indirectness of some of the comparisons. For applications of Table 1, however, we would recommend the adoption of the values headed '9*A*', which were obtained by direct comparison of the response of earphones on a B.S. artificial ear and a 9*A* coupler, respectively.

The work described was carried out as part of the research programme of the National Physical Laboratory, Teddington, and this article is published by permission of the Director of the Laboratory.

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THE RE-SURVEY OF TYPHOON EFFECTS ON JALUIT ATOLL IN THE MARSHALL ISLANDS

By DR. DAVID I. BLUMENSTOCK, DR. F. R. FOSBERG and CHARLES G. JOHNSON*

IN a previous issue of *Nature*[1], one of us (D. I. B.) reported briefly on the first findings of a field-party of seven scientists who visited Jaluit shortly after it was struck by Typhoon *Ophelia* on January 7–8, 1958. More recently, some detailed results of this initial survey have been given in an article by Dr. Edwin D. McKee[2] and in a series of papers by all seven participating scientists which is in the press[3].

Meanwhile, the present authors visited Jaluit Atoll during the period October 19–29, 1960, to observe in general what changes have occurred since the first survey, and especially to learn what effects have persisted most strongly and may long be expressed on the atoll. In this re-visit, sponsored by the Pacific Science Board of the National Academy of Sciences–National Research Council, the Office of Naval Research, and the Trust Territory of the Pacific Islands, we were greatly aided by Mr. Eric Knudsen, who is the resident agriculturist at Jaluit for the Trust Territory. It is the purpose of this article to report very briefly and in a preliminary sense on our findings during this re-survey.

General findings. The initial survey revealed that the typhoon had created rubble bars on the reef-flat on the ocean side of the eastern islets and had created other bars or extended existing bars on the lagoon reef-flats of some of these same islets. The typhoon had also laid down thick sheets of coral gravel across large sections of the eastern islets, had scoured out channel-ways, and had destroyed or felled 70–90 per cent of the trees throughout the eastern islets and 20–50 per cent throughout the western ones. Economically, the damage was so severe that the Trust Territory Government had to supply sufficient food to meet more than 60 per cent of the food requirements of the 1,200 native inhabitants.

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The re-survey has revealed that although the effects of the typhoon are still evident on all islets, there have been very significant changes. In virtually all locations studied the rubble bar on the ocean reef of the eastern islets has moved landward. In several places it has joined the main body of land ; in others the rubble has spread out to form boulder tracts on the reef-flat ; and in still others the bar remains separated from the main land body, but 20–50 yd. closer to it. New bars on the lagoon side of the eastern islets seem largely to have remained intact, though doubtless their form has changed somewhat. Gravel sheets still lie across the islets, but are being invaded by vegetation. In most places the vegetation has made considerable recovery. Culturally, re-planting of coconut palms has been virtually completed, some breadfruit have been replanted and the recovery of damaged or partially destroyed trees has greatly improved the local food supply. These three aspects of change—geomorphic, vegetational and cultural—will be discussed at somewhat greater length, and some comment made on renewal of the soil.

Geomorphic changes. The most conspicuous geomorphic change since Typhoon *Ophelia* has been the migration, removal or spreading out of nearly all of the rubble bars that were formed by the typhoon along the middle of the ocean reef on the eastern side of Jaluit. Remnants of the bar that do remain are little changed, except that there has been locally a slight increase in height by addition of gravel or decrease in height by removal of gravel and consequent slumping of the boulders and slabs that comprise the major part of the bars. Where the rubble bars have been removed, broad, low tracts of imbricated slabs and boulders remain. In a few places even these have been removed and the reef-flat is essentially bare. The materials derived from the bars now form gravel and rubble ridges or ramparts along the seaward edges of the islets, as was the situation before the typhoon.

Less change was noted in the off-shore bars along the lagoon side of the eastern reef. Essentially all the bars remain intact and show little or no loss of material or slumping. On the contrary, the bars have been enhanced in some places by addition of fresh gravel tossed up by waves in the lagoon.

Within the tide zone, scour pits and channels cut through sand or poorly consolidated sediments have become partly filled and will probably fill more. Little change could be seen in scour pits and channels at heights above the effect of tides and waves ; these are likely to become permanent irregularities in the topography until the next typhoon.

The general condition throughout Jaluit resulted from a re-adjustment of the shore-lines and bars created by the typhoon back to alignments of equilibrium approximating those that existed before the typhoon. Gravel and rubble bars, the situations of which are not too much at variance with the normal regimen of the waves, currents and winds, will probably remain in place long enough to become sufficiently indurated to be permanent reminders of Typhoon *Ophelia*. Other features that are likely to be more or less permanent are the gravel sheets that covered large seaward land areas of the windward islets, the scour channels, and the tidal ponds between the lagoon gravel bar and the islets.

Vegetation and soils. Superficially, the aspect of the vegetation has changed strikingly in the two and a half years since the 1958 survey.

Most of the areas that were then barren expanses of coral rock and gravel are now green and have a luxuriant, though low, herbaceous and shrubby vegetation. These areas, swept by both wind and high waves, still have little that could be called soil in the ordinary sense, as the fine material was generally washed out by the typhoon waves. Locally, only a strictly pioneer vegetation has become established, or even none at all. Elsewhere the pioneer species have largely been smothered by aggressive secondary vegetation.

Areas which were affected only by wind, rather than wind plus water, have, in many places, produced a dense low forest or tall scrub in which *Pipturus argenteus* is a principal component.

Estimation of the degree of recovery of the vegetation has been complicated by the fact that, during the extensive re-planting of coconuts, the brush has been largely cleared out once ; therefore the present vegetation represents recovery from this operation, as well as from the effects of the typhoon. The pre-typhoon vegetation was completely dominated over most islets by coconut and breadfruit groves and plantations. As pointed out in the 1958 article, these were in a large measure damaged or destroyed. Although re-planting, at least of coconuts, has been extensive, the planted seedlings have not yet assumed an important role in the vegetation, and it will be certainly 10–15 years before the more thoroughly devastated areas can be expected to resume their former densely forested appearance. Further, unless there is a greater emphasis on breadfruit planting than at present, the resulting forest will be far more dominated by coconut palms than was the case before the typhoon. Minor vegetation types, such as mangrove, *Pemphis* forest, beach ridge scrub and scrub forest and the *Pisonia* forest on small protected islets are mostly already beginning to resume their former aspects, and only need to grow up to their former stature to be much as before.

Much information was gained on the mode of adaptation of some of the important atoll and strand species to survival and recovery from typhoons. For example, of the tree species, *Pandanus*, *Cocos*, *Bruguiera* and *Pipturus* have largely replaced themselves by seedling reproduction, whereas *Pemphis*, *Artocarpus*, *Terminalia*, *Pisonia* and *Cordia* have conspicuously sprouted from the remains of plants damaged by the typhoon. *Scaevola* and *Tournefortia*, while sprouting abundantly from old stumps, have also appeared from seed in great profusion.

The difference in soils between wave-swept and non-wave-swept areas is conspicuous. In the former, whether the soils were buried by gravel or removed, little new soil development has taken place beyond the addition of some litter on the surface and some humus and roots in crevices and interstices. On the areas still retaining the original soil, the effect of the typhoon has perhaps proved beneficial. In places, large quantities of organic debris were added to the soil and much of this has decomposed; also, since the typhoon, the people have shown very little tendency to resume the previous burning of vegetable trash but rather they now allow it to rot. Consequently, many of the soils of the non-wave-swept islets are, at present, in excellent condition as coral atoll soils go, and are loose, friable, dark and rich in organic matter even when gravelly. This state of affairs may well continue so long as burning is not resumed. On most of the wave-swept areas soil amelioration will be much slower, depending on the weathering of gravel, addition of organic matter from root growth and leaf fall, and carrying in by wind or waves of sand- and silt-sized materials.

Cultural changes. We also noted marked changes in the cultural scene. Dwellings and other structures in the villages had all been repaired or rebuilt. Village walks had been cleared, and several have been bordered with stones. Where before there were none, ornamental plants now grow in neatly arranged gardens in front of several dwellings; and in many places coral debris has been cleared from garden areas and stacked in mounds nearby. The debris from fallen trees has been cleared from large parts of the inhabited islets, but not from the uninhabited ones.

There are now many more outrigger canoes than there were in April 1958. No count was made of these, but several boats were seen in each of the five villages visited (Jabor, Majuriruk, Pinlep, Jaluit, Imroj). None of these canoes exceeds 15 ft. in length, but we were told that they meet the needs of the natives for transport and fishing in the lagoon and at distances up to a few miles out to sea.

Among the subsistence crops that are now thriving and are being used are breadfruit, pandanus, pumpkins, bananas and sweet potatoes. The last two are especially widely grown. Coconuts remain the chief subsistence crop despite the large number of trees destroyed in the typhoon. In contrast, taro, which was an important crop on Pinlep Islet prior to the typhoon, is now only just beginning to be raised again.

The administration has sponsored and assisted with a wide-spread programme for the re-planting of coconut palms to provide a cash crop in the years ahead. This programme has been completed on all but the eastern islets, where it has almost been completed. At present, however, there is little or no copra produced for export, and the principal exports are trochus shell and handicrafts, neither of which provides a substantial cash income.

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[1] *Nature*, 182, 1267 (1958).

[2] *J. Sediment. Petrology*, 29, 354 (1959).

[3] *Atoll Research Bull.* No. 75 (1961).

OBITUARIES

Prof. D. Cordier

Physiologists throughout Europe will have learnt with sorrow of the death in October of Prof. Daniel Cordier in his sixtieth year. From 1946 he had held the chair of general physiology in the University of Lyon, but during the war years he was a well-known figure in Cambridge, where he conducted researches on chemical warfare for the French Forces under General de Gaulle.

Before the Second World War he had been in charge of the research station at Le Bouchet as director of the Physiological Laboratory under the Ministère de l'Armement, and in June 1940 he was one of the party of French scientists brought to England by the Earl of Suffolk to carry on their war work for the Allies. He and Madame Cordier lived in Cambridge until 1945, accepting exile with an unshaken faith in the outcome and making many friends.

Trinity College was particularly fortunate in having Prof. Cordier as a genial member of its society. His sturdy figure became a symbol of the French resistance, the colleagues who came to consult him were welcome guests at the high table, and his regard for the College added to the pleasure of his company.

He had an extensive knowledge of pulmonary and renal physiology which was of great assistance to all who worked in the Physiological Laboratory, and his own work on traumatic shock and intoxication showed his experimental skill and made a valuable contribution to war-time research.

After his return to France he continued and extended his work on more general lines, studying the effects of different forms of shock in a wide range of animals and dealing particularly with the disturbance of glycogen metabolism in the heart and liver. He became secretary-general of the Association des Physiologistes de Langue Française and the French representative in the International Union of Physiological Sciences. He will be greatly missed by the many colleagues who knew him in England and had the pleasure of seeing him again established in his own laboratory after the War.

Adrian

Mr. Henry Braid Irving, O.B.E.

Henry Braid Irving died in hospital at Stoke Mandeville, Bucks., on January 12 at the age of seventy-one after a lifetime devoted mainly to aerodynamic research. He was educated at the King's School, Chester, and at the Manchester College of Technology, and this was followed by four and a half years of practical and design experience in marine and general engineering on the Clyde.

He commenced his aerodynamic researches at the National Physical Laboratory in 1914, and his great contribution to the advancement of aeronautical science is indicated by the publication of more than sixty papers in the next twenty-five years. They cover a wide field, including airships, seaplanes, kite balloons, radiators, stress calculations and buffeting in addition to aeroplane stability and control problems and the spinning of aeroplanes. He is best known for his work on these last subjects, being the first to suggest the use of the slot for the improvement of lateral control in August 1922, a subject on which he published several papers; this was later recognized by the award of the Charles Wakefield Gold Medal by the Royal Aeronautical Society in 1929. The aerodynamic balancing of controls was aided by his proposal for a shrouded nose balance in November 1939, and a practical method of balancing split flaps published in May 1936 is known by his name.

In 1939 Irving was transferred to the Royal Aircraft Establishment and later became assistant director of scientific research (air) at the Ministry of Supply until his retirement in 1954. While at the Ministry he was responsible for initiating in 1950 researches on the reduction of the noise from jet engines. He wrote several papers on noise, was a consultant on this subject to the Ministry during 1954–57 and afterwards to the firms of Bristol-Siddeley and Westland Aircraft. He had also been chairman of the Noise Research Committee of the Aeronautical Research Council and a member of several of its other committees.

He was made a Fellow of the Royal Aeronautical Society in 1932, and was a founder member and chairman of the Man-powered Aircraft Committee of the Society, the committee which has fostered the growing interest in Britain in man-powered flight. Due to his personal efforts and his published articles, the prospects of man-powered flight in the near future has been enhanced.

Henry Irving was a man of great personal charm, kindness and sincerity, and he was an inspiration to all those who came in contact with him. He leaves a widow and two daughters. J. L. Nayler

Mr. W. A. Kay

William Alexander Kay, who died in January, was a prince of laboratory stewards whose fame spread far beyond the Physics Laboratory at Manchester in which he spent the whole of his working life. He started there as a laboratory assistant in 1894, and became steward in 1908, serving successively under Sir Arthur Schuster, Lord Rutherford, myself and Prof. P. M. S. Blackett. He was Rutherford's chief helper in the great days of his Manchester researches. In recognition of his services to the University he was made an honorary M.Sc. in 1946, and the University staff service centre is named after him.

He was an excellent steward and brilliant at devising experiments and demonstrating them in class. When Rutherford left Manchester in 1919 he invited Kay to go to Cambridge with him, but Kay could not bring himself to leave his native heath. How fortunate this was for the very inexperienced young professor who succeeded Rutherford can only be realized fully by myself. A great change has come over laboratories since those days, with their large establishments of secretaries, accountants, research and lecture room assistants and mechanics. Kay combined all these roles, and those of guide, philosopher and friend as well.

But it is not for his skill and his unflagging zeal that he will be chiefly remembered. His integrity, discretion, wisdom, warmth, and combination of modesty with a sturdy independence made a deep impression on all who knew him. The comparison may seem fanciful, but it is one which I have often made in my own mind. His personal qualities endeared him to us in much the same way that Michael Faraday's personality endeared him to his contemporaries. W. L. Bragg

NEWS and VIEWS

Dr. Jerome B. Wiesner

President Kennedy has appointed Dr. Jerome B. Wiesner to succeed Dr. George B. Kistiakowsky as special adviser for science and technology to the President. Dr. Wiesner has been on the staff of the Massachusetts Institute of Technology since 1946, being appointed to the grade of professor in the Department of Electrical Engineering in 1950. During the same period he carried simultaneous responsibilities at the Institute for the research laboratory of electronics, of which he became director in 1952. Born in Detroit, Michigan, in 1915, he went to the nearby University of Michigan, earning the degrees of B.S., M.S., and Ph.D. in engineering. During his twenties, he sampled a variety of occupations, ranging from a broadcasting station to the Library of Congress (as chief engineer). Thereafter he spent three war years at the Radiation Laboratory, Massachusetts Institute of Technology, followed by a year at the Los Alamos Scientific Laboratory during 1945–46. In addition to his academic and research posts, he has acted as consultant to industrial firms. Dr. Wiesner's research and scientific interests have been principally in the fields of acoustics, electronics, radar and theory of communications. Among his achievements was the development of the 'scatter' communication system, making possible the extensive distant early warning line spanning the northern perimeter of the American continent. In recent years, his interests in the international political problems of science have widened, stimulated in part by membership of President Eisenhower's scientific advisory committee, by his appointment in 1958 as staff director of the U.S. delegation to the Geneva Conference on means of reducing the dangers of surprise attack, and by his attendance at several Pugwash Conferences.

Machine Tool Industry Research Association :
Mr. A. E. de Barr

Mr. A. E. de Barr, who has been appointed the first director of the Machine Tool Industry Research Association, has had a distinguished career in industrial research. Born in 1918, he graduated with first-class honours in physics from the University of Leeds in 1939 and, after a short period of postgraduate research, proceeded to the Mine Design Department of the Admiralty. He remained with the Admiralty until the end of the War and then joined the firm of Joseph Sankey and Sons, Ltd., as research superintendent (physics). He transferred to the Guest, Keen and Nettlefold group of companies when the Research Laboratories of Sankey's became the Central Research Laboratories of the group. In 1950 he accepted the position of leader of the Physics Division of the Research Laboratories of Elliott Bros. (London), Ltd., where he was responsible for the establishment and development of physics, chemistry and metallurgy laboratories. Mr. De Barr joined the staff of the Shirley Institute, Manchester, in November 1953 as head of the Spinning Department, where his main contribution has been in the application of physical concepts to fibres and yarns and the engineering improvement of the spinning process. He is the author of a number of scientific papers on physical and engineering subjects, a Fellow of the Institute of Physics and has for many years played an active part in the development of technological education. The new Research Association is to be congratulated on getting as its first director a man of outstanding scientific ability, who brings to it also an appreciation of the problems of industry, and particularly of engineering industry, at a time when the machine tool industry in Britain looks to be moving into a new phase of production techniques.

Pure Mathematics at Swansea :

Prof. R. Wilson

PROF. R. WILSON, head of the Department of Pure Mathematics in Swansea, retires in September 1961 after being a member of the staff of the University College of Swansea (University of Wales) for forty years. Educated at Bradford Grammar School and Queen's College, Oxford, where he was a Hastings Scholar, he served in the First World War before graduating in 1921. In the same year he was appointed an assistant lecturer in the Department of Mathematics in Swansea, a year after the foundation of the College. He became a senior lecturer in 1929 and professor from 1942. His published research papers cover a variety of topics in mathematical analysis and those on the classification of singularities of functions of a complex variable are perhaps best known. He spent a year in Cambridge as a Leverhulme Research Fellow during 1938–39, and in 1955 was awarded an Oxford D.Sc. Three times dean of the Faculty of Science, and for his last five years the senior professor in Swansea, Prof. Wilson has served at one time or another on a large number of the committees of the College and of the University of Wales, and has often provided a powerful stabilizing influence, in days of rapid expansion and frequent change, using his long experience to help preserve continuity with the earliest days of the youngest of the Welsh university colleges.

Prof. J. D. Weston

DR. J. D. WESTON, reader in mathematical analysis in the University of Durham at King's College, Newcastle upon Tyne, has been appointed to succeed Prof. Wilson. Among his responsibilities will be that of organizing all the post-intermediate teaching of mathematics to applied scientists in the College, and it is of interest in this connexion that Prof. Weston first graduated by taking a first-class honours degree in engineering in London in 1939. As a practising radio development engineer, he was on the staff of Standard Telephones and Cables, Ltd. After gaining a London B.Sc. in mathematics (1944) and Ph.D. in engineering (1946), he was for a short time lecturer in mathematics at the Woolwich Polytechnic and later lecturer in the Mathematics Department of the University of Sheffield until 1949. He then moved to King's College, Newcastle, where he became a senior lecturer in 1958 and was appointed to a personal readership in 1960. During the academic year 1959–60, he held a visiting associate professorship in the California Institute of Technology, and in 1960 was awarded a London D.Sc. Prof. Weston has a distinguished research record and an international reputation as a functional analyst. His published work has been concerned mainly with the properties of certain topological spaces, including linear spaces, and more recently with an elegant treatment, based on convolution algebra, of that part of distribution theory from which a rigorous account of the Heaviside operational calculus can be developed.

Re-organization in the U.K. Atomic Energy Authority

CHANGES in the organization of the United Kingdom Atomic Energy Authority are being made, as the result of recommendations of the Select Committee on the Industrial Group which reported in July 1959. Sir William Penney has been appointed deputy chairman, with primary responsibility for scientific and technical co-ordination throughout the Authority. The work of designing and developing nuclear reactors will be concentrated, to the greatest possible extent, in one group of the Authority. The major development facilities required for this purpose will be under its control. The structure of the Development and Engineering Group will be modified for this purpose. It will be re-named the Reactor Group and will be responsible for the design and development of reactors, and for relations in this field with industry in Great Britain and with overseas countries. The Winfrith Research Establishment, which has responsibilities in the same field, will be transferred from the Research Group to the Reactor Group. The existing responsibilities of the Development and Engineering Group for the design and construction of plant, works and buildings will be transferred to a new Engineering Group, which will also take over from the Production Group its responsibilities for the design and inspection of fuel elements for production purposes. Apart from the transfer of the Winfrith Research Establishment, the structure and responsibilities of the Research Group remain unchanged. The Weapons Group is unaffected by the new arrangements.

National Lending Library for Science and Technology

IN a written answer in the House of Commons on February 16, the Parliamentary Secretary for Science, Mr. D. Freeth, said that from April 1957 until the end of March 1961, expenditure on the National Lending Library for Science and Technology was estimated at £904,000, including the capital cost of adapting the buildings at Boston Spa, Yorkshire. The net cost of the existing Library unit in London would be about £272,000 in the current financial year, and after the move to Boston Spa was completed and the Library was fully operational, at the end of 1962, the annual cost would be about £315,000. No direct expenditure had yet been incurred on the National Reference Library for Science and Invention.

The Health Programme in India

THE November issue of *Science and Culture* (26, No. 5) contains an article outlining the health programme of the Third Five Year Plan in India, and also a discussion of the proposals by Dr. A. N. Khosla, delivered to the Health Panel of the Planning Commission. Of the 300 crores of rupees allocated for health, 80 crores are for water supply and sanitation, 50 crores for primary health units, hospitals and dispensaries, 92 crores for control of communicable diseases, 38 crores for education and training and 25 crores for family planning. The shortage of medical personnel is a grave problem; there are about 71,000 doctors in India, 25 per million in rural areas and 800 per million in urban areas, while the present 55 medical colleges have an annual intake of 5,000 and train about 2,800 graduates each year. The position regarding women doctors is particularly unsatisfactory. It is suggested that it is not suitable to increase the number of medical colleges at present, but that the resources of the Plan could be more profitably used in raising the standard of teaching, providing more equipment and space in the hospitals and laboratories and increasing the staff of qualified teachers in existing institutions. The total allocation of Rs.300 crores is regarded as inadequate, and besides a determined effort to eliminate the adulteration of food, a bolder policy in regard to water supply and sanitation is urged, and expansion of the existing scheme for eradication of endemic goitre by the supply of iodinated salt is recommended to cover the whole of the endemic belt. The public health laboratories envisaged should be provided with free diagnostic services for the public.

Information on Translations

A NEW international information office has been jointly established by Euratom, the U.S. Atomic Energy Commission and the U.K. Atomic Energy Authority in Brussels. Called 'Transatom', it is intended to act as a clearing house for information on the translations of nuclear literature available from a variety of organizations. It publishes monthly the *Transatom Bulletin*, which lists in the style of *Nuclear Science Abstracts* translations at present available of articles, with source and price. It also lists forthcoming translations as well as translations of whole journals. In addition to this service, 'Transatom' is assembling a master file of translations of interest. This file may be consulted by subscribers to the *Bulletin*. The first issue of the *Bulletin* is dated December 1960 (Pp. 80. 50 francs; 1 dollar. Bruxelles: Euratom, Transatom Service, 51 rue Belliard, 1960). The majority of the translations listed are from Russian and Japanese journals, and the language of the translation is English, French or German. Further information may be obtained from Euratom, Transatom Service, 51 rue Belliard, Brussels.

International Natural History Research

YOUNG naturalists all over Europe have been carrying out a survey of the arrival and departure of the swift and the flowering time of the wood anemone. Their report for 1959 has been issued by the International Youth Federation for the Study and Conservation of Nature. The first swift was reported in Holland on April 18, and the 'last first' in Finland on June 7. On the return migration the swifts disappear from the more southerly breeding localities before they have left those in Scandinavia. The first wood anemone came into bloom in Holland on March 19, and the 'last first' in mid-Finland on May 18. Many Nature clubs in Britain are associated with the Federation through the British Co-ordinating Group, but there is no reason why young naturalists in any society should not participate. The programme for 1961 will follow the plan as used in previous years. Introductory notes and duplicated recording sheets will be available very soon and the work will begin in the early spring. Recording for the wood anemone will be over by about the beginning of April, but recording for the swift will continue until the end of the summer. For the swift, the dates for the arrival of the first individual and the main flock will be required, details about nesting sites, numbers of young birds in selected nests and also the dates at which the birds left the area in the autumn. For the wood anemone, information will be required about the habitat, abundance of plants at a given locality, details of the phenology and also counts of the number of petals on individuals in a random sample. Information and recording sheets will be available from the secretary of the *Naturalist*, Derek S. Davis, 56 Altmore Avenue, East Ham, London, E.6.

The National Art Gallery and Dominion Museum, New Zealand

THE report of the Board of Trustees of the National Art Gallery and Dominion Museum for the year ended March 1960 discloses a year of activity in many directions (Pp. 30. (H. 21.) Wellington: Government Printer, 1960). Temporary exhibitions included an unusual one of Czechoslovakian Truka puppets. The re-appointment of an educational officer has proved of great value to this side of the gallery work. In the Museum section some valuable field work has been undertaken including natural history investigations in twelve outlying islands in Cook Strait and the recording of Maori occupation sites in the Wellington District. An impressive list of publications by the staff is an indication of the value of research as one of the functions of a museum.

Coronary Artery of the Portuguese East African

MYOCARDIAL infarction is generally recognized to be the consequence of thrombosis in a diseased coronary artery. While not all diseased arteries promote thrombosis, the frequent association of thrombosis and coronary disease has led to the view that perhaps an understanding of the factors promoting disease of the coronary artery may assist in preventing the high incidence of myocardial infarction in the Western world. In searching for aetiological factors, attention has been focused largely on the age group in which coronary disease is most likely to declare itself clinically. Equally important, but less well known, is the observation that the coronary artery is unique in that regressive changes are initiated early in life, and even within a month after birth. Some of these regressive changes, if accentuated, are unmistakably similar to those which have been described in advanced sclerosis, with and without atherosis of the coronary artery.

During the past twenty-four years, several reports have directed attention to the rarity of coronary thrombosis in the indigenous peoples of different parts of Africa. While advanced coronary disease has been described in the African, it is believed that the disease is less common, milder in character and declares itself later than in the white races of Europe and the United States of America. In view of this marked difference in susceptibility to coronary artery disease of white people and Africans, and of the expressed opinion that the regressive changes occurring in the coronary artery soon after birth may be linked to the pathogenesis of coronary disease, Joseph Gillman, Christine Gilbert and Manuel D. Prates have considered it desirable to examine the coronary arteries at different periods of life of an African population in which coronary artery disease is known to be uncommon (*S. Afr. J. Med. Sci.*, 25, Nos. 2 and 3; September 1960). They have endeavoured to record: (1) the histological reactions observed in the coronary arteries at successive age periods from birth to more than sixty years in the Portuguese East African in Lourenço Marques; (2) to type the reactions in a way which will make future comparison between these and reactions in the coronary arteries of peoples elsewhere in the world; and (3) to assess the significance of these reactions for the development of severe coronary artery disease and thrombosis.

Distribution of British Reptiles and Amphibians

A PAPER by R. H. R. Taylor on the distribution of Reptiles and Amphibians in the British Isles was first published in the *British Journal of Herpetology* in June 1948 (1, No. 1). In this paper, a complete record of the occurrence of the different species in the British Isles and Channel Isles was presented. It is proposed to re-write the paper and include records up to the end of 1960. To obtain a complete and accurate picture of the present distribution, up-to-date records from all areas are required. For 40 or 50 years there have been no published records of even the commonest species from a number of vice-counties. Lieut.-Colonel Taylor is anxious to obtain information about the following species: *Anguis fragilis* (slow worm); *Lacerta vivipara* (viviparous lizard); *Lacerta agilis* (sand lizard); *Coronella austriaca* (smooth snake); *Vipera berus* (viper or adder); *Natrix natrix* (grass or ringed snake); *Triturus helveticus* (palmate newt); *Triturus cristatus* (great crested or warty newt); *Triturus vulgaris* (smooth newt); *Bufo calamita* (natterjack); *Bufo bufo* (common toad); *Rana temporaria* (common frog); *Rana ridibunda* (marsh frog); and *Rana esculenta* (edible frog). The records should show the year the species were observed and the locality. Any data should be sent to Lieut.-Colonel R. H. R. Taylor, The Studio, Belchamp St. Paul, Sudbury, Norfolk.

Dependence of Photosynthesis on Temperature in Higher Plants

IN a paper entitled "Studies of Climatic Ecotypes in Higher Plants: the Temperature Dependence of Apparent Photosynthesis in Different Populations of *Solidago virgaurea*", O. Bjorkman, C. Florell and P. Holmgren (*Kungl. Lantbruckshögskolans Annaler*, 26, 1; 1960) present results of a study of the temperature dependence of apparent assimilation in plants from different populations. Clonal material was obtained from different climatic zones, ranging from Skåne in south Sweden to Finmark in north Norway, and grown in a phytotron in chambers maintained at three different climates (alpine, continental and maritime). The climates reproduced in the chambers were based on 20-year meteorological records. Some populations were also grown under constant day and night temperatures (18° C. and 8° C. respectively) with a photoperiod of 18 hr. The apparent photosynthesis of intact plants was measured. The ecotypes were found to vary in chlorophyll content, but these differences were not correlated with values of apparent photosynthesis. The optimum temperature for photosynthesis of plants from a maritime habitat in south Sweden was 20° C., irrespective of the climate in which they were grown. Plants from an alpine region in north Norway, on the other hand, with a few exceptions, had an optimum temperature of 16° C. in all three climates. By contrast plants from an inland region of central Sweden were more variable. The results show a good correlation with the temperature conditions during the growing season of the three localities, and there appears to be a physiological adaptation to the temperature conditions which is under genetical control. With plants grown under constant day and night conditions some differences were found, but not as obvious as those in the climate series.

Synoptic Auroral Maps

AT the Antarctic Division of the Department of External Affairs, Melbourne, Major F. R. Bond and Dr. F. Jacka have undertaken the compilation of synoptic maps showing, at quarter hourly intervals, the plan position of auroras observed in the southern hemisphere during the International Geophysical Year. So far, only the data from Australia and the antarctic stations of Australia, France, Belgium and New Zealand have been plotted. With such maps it has been possible to make an estimate of the location of the isoaurore of maximum frequency of occurrence of auroras (*Aust. J. Phys.*, 13, 610; 1960), and to make a general study of the development of auroral displays and associated magnetic disturbance on the equatorwards side of the auroral zone (*Aust. J. Phys.*, 13, 477; 1960). But on some occasions of intense magnetic disturbance ($Kp = 8$ or 9) the aurora, for many hours, takes the form of an extensive red 'diffuse surface' over polar-cap stations. At present the synoptic maps are not complete enough to establish the extent of these 'surfaces' nor are any measurements of their height available. However, it is thought that on these occasions the electric current which gives rise to magnetic disturbance is in the nature of a continuous sheet rather than of filamentary structure as seems usual (*Aust. J. Phys.*, 14; March 1961). G. Weill and Mlle. S. Huille, of France, and Mlle. P. Doyen, of Belgium, have co-operated with Messrs. Bond and Jacka in the task of compiling these maps, and the co-operation is now invited of other workers concerned with the study of southern hemisphere auroral data.

Engineering Aspects of Lubrication

THE Imperial College of Science and Technology is organizing a short postgraduate full-time course on "Engineering Aspects of Lubrication" during May 1–19. This will be an intensive course covering the subject from the engineering aspect, including theories of fluid film, thin-film lubrication, wear of lubricants, lubricating materials, the properties of natural and synthetic oils, etc. The number of participants for the course will be limited to thirty-two and the fee will be 25 guineas. Further information can be obtained from the Registrar, Imperial College of Science and Technology, London, S.W.7.

The British Institute of Radiology: Annual Congress

THE annual congress and exhibition of the British Institute of Radiology is to be held jointly at the Central Hall and Church House, Westminster, London, W.C.1, during April 26–28. A number of symposia are to be included in the programme: "Localization using Physical Devices, Radioisotopes and Radiographic Methods" (April 26, at the Central Hall); "The Age Factor in the Susceptibility of

Man and Animals to Radiation" and "Planning of New Radiotherapy Departments" (April 27, at the Central Hall); "Planning of New Diagnostic Departments" and "Radiological Assessment of Bone Density" (April 27, at Church House); "Recent Developments in Linear Accelerators for Therapy" (April 28, at the Central Hall); "Industrial Developments in Diagnostic Equipment" (April 28, at Church House). The Silvanus Thompson Memorial Lecture will be given by Sir Bernard Lovell on "The Condition of Outer Space", at the Central Hall on the evening of April 26. Two exhibitions are also being arranged, one at the Central Hall and the other in the Hoare Memorial Hall, Church House. Further information can be obtained from the General Secretary, British Institute of Radiology, 32 Welbeck Street, London, W.1.

The Night Sky in March

Full moon occurs on Mar. 2d. 13h. 35m. U.T. and new moon on Mar. 16d. 18h. 51m. The following conjunctions with the Moon take place: Mar. 1d. 14h., Regulus 1° N.; Mar. 12d. 20h., Saturn 3° S.; Mar. 13d. 00h., Jupiter 3° S.; Mar. 22d. 04h., Aldebaran 0·2° S.; Mar. 24d. 18h., Mars 7° N.; Mar. 28d. 21h., Regulus 1° N. There will be a partial eclipse of the Moon on March 2, invisible at Greenwich. Mercury is too close to the Sun for observation. Venus is an evening star, favourably placed for observation. Venus sets at 21h. 50m., 21h. 35m. and 20h. 30m. on March 1, 15 and 31, respectively, about four hours after the Sun at the beginning of the month. Venus attains its greatest brilliancy on March 5, when its stellar magnitude is −4·3. During the month its distance from the Earth decreases from 44 to 27 million miles and the visible portion of the apparent disk from 0·315 to 0·045. Mars sets at 4h. 20m., 3h. 40m. and 2h. 55m. at the beginning, middle and end of the month, respectively. Mars is conveniently situated for observation. It is at a distance of 104 million miles from the Earth on March 15, when its stellar magnitude is + 0·6. Jupiter and Saturn are too close to the Sun for easy observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Mar. 21d. 21h. 11·5m., γ Tau. (*D*); Mar. 29d. 23h. 45·5m., 59 Leo. (*D*). *D* refers to disappearance. The spring equinox occurs on Mar. 20d. 21h.

Announcements

Lord James of Rusholme, High Master of Manchester Grammar School since 1945, has been appointed as the first Vice-Chancellor of the University of York; he is expected to take up his duties early in 1962. Lord James, who is a science graduate of Oxford, was a member of the University Grants Committee during 1949–59.

Dr. Christopher E. Barthel, jun., assistant director of the Armour Research Foundation of the Illinois Institute of Technology, has been appointed to the Office of Special International Programmes of the National Science Foundation, Washington, where he will be programme director for foreign science activities.

Mr. R. P. Newman has been appointed head of the Members' Service Department of the British Welding Research Association in succession to Mr. P. H. R. Lane, who has been appointed director of research of the Drop Forging Research Association. Mr. T. R. Gurney will succeed Mr. Newman in charge of fatigue researches at the British Welding Research Association.

Dr. C. G. Hunter, formerly professor of physiological hygiene, School of Hygiene, and associate professor, Banting and Best Department of Medical Research, University of Toronto, has been appointed director of the Tunstall Laboratory, near Sittingbourne, Kent, recently opened by 'Shell' Research. The laboratory will be concerned with the possible effects on man and other vertebrates of contact with products being developed by the Royal Dutch/Shell Group of Companies, and has four divisions, dealing with pathology and pharmacology, physiology, chemistry and biochemistry, respectively.

The Ninth German Plastics Convention is to be held in Berlin during April 12–14. Further information can be obtained from the Arbeitsgemeinschaft, Deutsche Kunststoff-Industrie, Karlstrasse 21, Frankfurt am Main.

The Plant Phenolics Group is holding a meeting on "The Importance of Plant Phenolics in Industry" at Cambridge during April 10–12. Further information can be obtained from the Honorary Secretary, Low Temperature Research Station, Downing Street, Cambridge.

The West German Starch Research Station, Detmold, is organizing an international conference on "Starch" at the Cereal Research Association, Detmold, during April 26–28. Further information can be obtained from W. Kempf, Cereal Research Station, P.O. Box 23, Detmold, West Germany.

The National Vegetable Research Station Association is holding a members' day on March 1. The subject of the day's programme will be "Soil Physical Conditions affecting Plant Growth". Further information can be obtained from Mr. G. Lesson, National Vegetable Research Station, Wellesbourne, Warwick.

The annual conference, organized by the Stress Analysis Group of the Institute of Physics and Physical Society, will be held in the University of Southampton during April 24–27. The topic for the conference will be "The Mechanics of Rupture". Particulars of the meeting can be obtained from Mr. L. Lawrence, Institute of Physics and Physical Society, 47 Belgrave Square, London, S.W.1.

The Easter congress of the Universities Federation for Animal Welfare is to be held at the University of Liverpool Veterinary Field Station, Leahurst, Neston, Wirral, Cheshire, during April 10–14. The programme will include lectures, discussions and visits connected with the Federation's work and with animal welfare in general. Inquiries should be sent to U.F.A.W. (Easter Congress), 7a Lamb's Conduit Passage, London, W.C.1.

The anniversary meeting of the Chemical Society is to be held in Liverpool during April 11–14. The meeting will include two concurrent symposia, namely, "Developments in the Chemistry of Boron Compounds" and "Some Aspects of the Chemistry of Natural Products", and also a number of visits. The meeting, other than the annual general meeting (April 13), will be open to the public. Further information can be obtained from the General Secretary, Chemical Society, Burlington House, London, W.1.

WESTERN DEPENDENCE ON OIL

A WARNING that Britain's dependence on oil has become "potentially dangerous to our national economy" is made by the 11,000 strong Association of Scientific Workers in a 'policy statement' in a new publication entitled *A National Fuel and Power Policy**.

The recommendations in this statement are put forward in the form of a twelve-point plan that has as a main objective improved co-ordination and development of the national resources of fuel and power. The objectives are worthy ones, and are set out in an attractive manner with some sound supporting arguments, but some of the means to be employed in the plan are open to question on the grounds of practicability.

The first point made is that the efficiency of the production of coal should continue to be improved with the view of making coal cheaper in real terms. For this purpose increased expenditure on scientific research is rightly regarded as necessary, but, in the subsequent recommendations, difficult and almost intangible problems are raised as to the maintenance of markets over fixed periods, the subsidizing of specific uses of coal, the 'firm basing' of the generation of electricity on coal and back-pressure generation by industry and much additional planning. Several of the suggestions made for controlling the balance of fuels and the types of appliance installed embody a measure of compulsion either by legal requirements or by discriminatory taxation. There is nothing intrinsically against this, though it is clearly contrary to the present Government's policy. Attempts to control markets in the manner suggested are not necessarily in the national interest, nor do they actually work out in the manner intended in practice. The back-pressure generation of power in industry is only economical if the individual circumstances of the case in point are favourable. Each set of circumstances becomes almost a law unto itself, and sweeping decisions as to their application in general cannot be made.

As regards the recommendation for the formation of a National Coal Chemical Board, it is not clear what advantages would accrue from yet another national board in a relatively small field of activity.

Concerning the needed improvements in research and development in the mining, utilization, and marketing of coal, controversy may well arise. Thus it is perhaps surprising to read that it may be necessary to abandon the conception of the British Coal Utilization Research Association as the National Coal Board's agent in the field of coal utilization, for it seems perfectly clear that the National Coal Board has always interpreted literally its remit under the Nationalization Act as "making supplies of coal available, of such qualities and sizes, in such quantities and at such prices, as may seem to them best calculated to further the public interest in all respects . . ."; in other words, utilization has not been the National Coal Board's responsibility under the Act; in fact the support given to the British Coal Utilization Research Association has been somewhat incidental. In point of fact this support has approximated to £140,000 per annum since nationalization, the overall income of the British Coal Utilization Research Association in 1959 being £427,000. The Association is thus operating on a much broader front than is implied in the statement of the Association of Scientific Workers. This research work covers contract work for the Atomic Energy Authority as well as that for the Ministry of Power. As regards the latter, the contract work on the slagging gasifier, as well as other features of the research programme, received commendation in the recent report of the Wilson Committee on Coal Derivatives. Further, the Association's real potential for work in coal utilization on a broad front was indicated, given adequate financial support.

It is not realized fully, perhaps, by the public that scientific work on the winning, preparation and use of coal requires an array of top-level scientists and technologists not yet even contemplated; moreover, to attract such personnel to-day to an industry in disfavour with the public mind will necessitate a radical change of approach. The expenditure on research by the National Coal Board, as given in the Wilson Report, was £2,427,300 in 1959, its turnover, £850 million, that is, approximately 0·29 per cent.

Cogent criticism is devoted to the world role of the oil industry, which is regarded as an international one dominated by a few large producers with price-fixing arrangements "typical of a cartel". "In Western Europe the price of oil and refined products has been below internationally arranged prices", the statement declares, and this increasing dependence in Western European countries on oil has made them vulnerable to price increases which would "adversely affect our position in world markets since fuel costs are a significant part of production costs". This may be true, but the question is not answered as to whether a manufacturer who finds the use of oil more economical than alternative fuels, mainly the derivatives of coal, should hold off conversion of his plant to oil because of this possibility. It is, however, evident from the arguments put forward that the challenge of oil can mainly be met by a substantial increase in technological activity in respect of coal.

A point of considerable interest is made as regards the inflexibility of the fuel oil/petrol ratio of refinery production in Britain. Thus Britain imports 73 per cent of its oil as crude oil, and this is refined so as to yield 27 per cent motor- and aviation-spirit, and 43 per cent fuel oil and other products such as paraffin. Refining could be adjusted, as in the United States, to yield 50 per cent of petrol and 17 per cent fuel oil. Such a change in refinery technique in Britain would result in British petrol requirements being met by half the present import of crude oil. Again, the trend towards oil is largely based on the comparison between modern oil-fired appliances and obsolete hand-fired coal-burning appliances. The advantages of oil-burning are certainly sometimes more apparent than real. What would be the position if a cheap industrial gas made by total gasification from coal were made available ? The advocacy of this development is strongly supported; indeed a call is made for a higher priority in the pursuit of this subject. It is believed, too, that industry would be bound to respond to its solution for manifold reasons. Taking the long view, as regards the future of the coal fields, this could well be an almost universal method for the utilization of coal, for it is the only foreseeable solution at present of the sulphur problem.

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* *A National Fuel and Power Policy*: a Policy Statement by the Association of Scientific Workers. Pp. 20. (London: Association of Scientific Workers, 15 Half Moon Street, W.1, 1960.) 1*s*.

R. J. SARJANT

BIOCHEMISTRY OF HUMAN TUMOURS

DURING November 17–19, an international conference on the biochemistry of human tumours was held at the University of Wisconsin, Madison. This meeting was sponsored by the Biochemistry Committee of the Cancer Chemotherapy National Service Center, National Cancer Institute, U.S. Public Health Service, with the present writer serving as programme chairman. Those invited to attend included five scientists from Europe and fifty-five from the United States. Among these were pathologists, clinical cancer chemotherapists, biochemists and biologists. The need for this conference arose from the conviction of members of the Biochemistry Committee, expressed in a guest editorial (James F. Holland and Charles Heidelberger, "Human Cancer, the Primary Target", *Cancer Research*, 20, 975–6; 1960), that despite formidable technical difficulties, increased biochemical research must be carried out on human tumours. This should include studies on the enzyme patterns of human tumours as compared with normal tissues, as well as research on the metabolism and mechanism of action of active tumour-inhibitory drugs in the human cancer patient and *in vitro* with specimens obtained at surgery or by biopsy.

The meeting was opened by James F. Holland (Buffalo, U.S.A.), who pointed out some of our major deficiencies in knowledge about human cancer. He directed attention to the variability of human tumours, even of the same kinds, with respect to growth-rates and patterns, and to their response to chemotherapeutic agents. Even though extensive work has been done on biochemical mechanisms of action of various antimetabolites in susceptible and resistant transplanted rodent tumours, similar work on human tumours is almost totally lacking. Moreover, there is little information on enzyme activity in human tumours, and on the effects of tumours on host metabolism, as exemplified by terminal cachexia. Holland felt there is a particular need to develop new methods to measure the growth-rates of inaccessible tumours and to get a measure of the amount of stromal and necrotic contamination of tumour specimens.

A brief review of human tumour pathology was given by Paul Kotin (Los Angeles), who showed a number of slides to illustrate diversity even among similar types of human tumours. He made the point that there is little, if any, correlation between the histological appearance of tumours and the carcinogenic stimuli that produced them. Therefore, he suggested that biochemical patterns reflecting essential consequences of the carcinogenic process might be studied more meaningfully in pre-neoplastic tissues, such as carcinomas *in situ*, rather than in the fully developed and highly diversified tumours. He also suggested that work be carried out to understand the differences in natural history between certain tumours in children and their counterparts in the adult. The morphological considerations were carried to another order of resolution by Francoise Haguenau (Paris), who discussed the electron microscopy of human tumours. She demonstrated with elegant slides the diversity of human tumours with respect to the appearance of the submicroscopic cellular organelles. Furthermore, some cells that appeared similar in the light microscope were found to differ markedly in their ultramicroscopic morphology, and she was able to distinguish on this basis between the α- and β-cells of Caspersson.

A. R. Curreri (Madison) discussed the surgeon's role in the collection of surgical specimens, and emphasized the willingness of surgeons to collaborate in co-operative enterprises provided that the purpose of the research was made clear. Much discussion followed, which was summarized by Holland: "Know thy surgeon and love thy biochemist".

A detailed discussion of the techniques that have been developed for low-temperature storage, with maintenance of viability, of cells grown in tissue culture was given by Cyril Stulberg (Detroit). The application of these methods to the storage of sections of human tumours, which would be essential in any proposed large-scale co-operative studies of tumour enzymology, remains to be worked out.

Because of the extreme heterogeneity of specimens of human tumours, it is clear that micro- and ultramicro-methods are required in most cases in order to obtain meaningful information. Accordingly, David Glick (Minneapolis) and Oliver Lowry (St. Louis) discussed in some detail the elegant and precise techniques that they have developed for the quantitative assay of enzymes extracted from samples the size of common histological sections and by microdissection. These involved mainly colorimetric, spectrophotometric, and fluorimetric methods. Another technique for enzyme assays with very small samples was described by V. R. Potter (Madison), involving the use of labelled substrates of high specific activity, paper chromatography, and the use of automatic recording equipment for the direct determination on the paper strip of the radioactivity in the substrate and product.

His extensive work on the amounts of some twenty enzymes in 800 human tumours by various histological staining methods was described by R. G. J. Willighaghen (Leyden). An enormous diversity of results was found; some enzymes were present in some tumours and absent in others from the same organ and of the same histological type. However, in some cases it has been possible to predict the clinical behaviour of tumours based on histochemical observations. Using similar techniques, L. Wattenberg (Minneapolis) reported that aminopeptidase, an enzyme not found in the normal gastric mucosa, occurs in many gastric malignancies, and in this metabolic feature resembles intestinal metaplasia of the stomach and also the mucosa of the small bowel.

James Danielli (London) pointed out that no single variable has been found in tumours to serve as a basis to design new drugs. Therefore it is necessary to summate small differences in order to obtain responses. He discussed his interesting research on the Walker 256 rat carcinosarcoma with aromatic nitrogen mustards containing a urethan group, which require enzymatic cleavage by the tumour before the mustard becomes active. He was able to demonstrate that feeding a chemotherapeutically inactive phenylurethan derivative to rats raised the level of the cleavage enzyme in the tumour, which resulted in a potentiation in chemotherapeutic effectiveness when the substituted mustard was given afterwards. This approach of inducing an enzyme in tumours that would liberate the active drug from a non-toxic precursor was discussed extensively.

Henry Pitot (Madison) described his work (in collaboration with V. R. Potter) on the enzymatic constitution of a series of transplanted rat hepatomas as compared with that of normal and regenerating liver. A number of marked differences were found, and enzymological evidence was presented to suggest strongly that the Novikoff hepatoma arose from bile-duct cells, whereas the Dunning hepatoma arose from parenchymal liver cells. In the case of the Morris 5123 hepatoma no qualitative difference between the tumour and liver was found in assays of some thirty different enzymes. However, several enzymes in liver that show marked variations in response to substrate administration and dietary changes showed no such response in the 5123 hepatoma under similar conditions. This may indicate that some biochemical control mechanisms may be lacking in this tumour.

A study, by conventional biochemical techniques, of about twelve enzymes and six analytical parameters was reported for twenty-three human tumour specimens by George Boxer (Rahway, New Jersey). He stressed the diversity and heterogeneity of the samples as examined by various criteria and the necessity of standardizing rigorously the enzymatic assays and determining stability under the frozen storage conditions employed in this work. The difficulty of deciding the basis to which the enzyme activity should be referred (wet weight, protein, deoxyribonucleic acid, etc.) was discussed in some detail. He and several others have used an analysis for collagen as an indication of the amount of connective tissue contaminating the tumour samples, but this has not as yet proved satisfactory.

C. de Duve (Louvain) discussed a strategic approach to chemotherapy by classifying the various enzymes that affect or are affected by a given drug. The target enzymes are acted on by the drug: a positive target would be the release or activation of enzymes that might kill cells, such as lysosomes; a negative target would be an enzyme which if inhibited would result in cell death. Auxiliary enzymes were defined as those acting directly on the drug; a positive auxiliary would convert the drug into a more active form; a negative auxiliary would detoxify the drug. It was agreed that this would be a useful way to consider mechanisms of drug action. de Duve also believed that it would be more useful to study a few enzymes very completely in human tumours than to carry out a superficial survey of the amounts of many enzymes.

At this point there followed thirty-two brief presentations by the remainder of the participants in the conference. These ranged from the purely conceptual to the presentation of potentially useful techniques, and stressed work on the mechanism of action of various chemotherapeutic agents in human beings. An open discussion followed.

The meeting was marked by a congenial spirit established among the participating pathologists, surgeons, clinicians, and biochemists that augured well for the future of this type of research, which requires close co-operation and mutual understanding among workers. The pall cast over the conference by the pathologists' demonstration of the tremendous heterogeneity and diversity of human tumours was never completely dispelled, however, and the most urgent problem to be solved before productive work can be done is to develop quantitative methods to cope with this situation, so that meaningful criteria can be established. It was agreed that all work should be done under strict histological control. The majority of the participants felt, with some vigorous dissenters, that this work would have to be carried out on a small scale in order to produce meaningful results. Some expressed the view that overall metabolic studies in cellular systems, particularly when dealing with mechanisms of drug action, might be more useful than enzyme assays, but all agreed that more work with human tumours was needed.

On the day following the conference, a small meeting of some of the participants was held to discuss organization of research in this field. It was agreed that informal study groups would be set up among the participants of the conference to discuss, by correspondence and afterwards by informal meetings, three general areas considered to be of crucial importance at this time. The first will deal with the problem of the diversity and heterogeneity of human tumours and the development of suitable techniques and a basis for meaningful biochemical studies. The second will involve asking biochemists interested in the enzymology of tumours to make recommendations as to which enzymes would yield most fruitful results on investigation. The third will deal with the stimulation of additional research on mechanisms of drug action in human cancer patients and *in vitro* in surgical and biopsy specimens of human tumours. Initially these activities will be co-ordinated and sponsored by the Cancer Chemotherapy National Services Center. Investigators who wish to have additional information or wish to participate in this programme are invited to communicate with Dr. J. Leiter or Dr. S. Schepartz at that agency.

The challenge of research with human tumours is one that is worthy of the interest, ingenuity, and participation by all scientists interested in cancer, working as individuals or as interdisciplinary teams. In order for this challenge to be met adequately a mobilization of scientific talent all over the world is required. The meeting recently held in Madison was a modest step in this direction. It is to be hoped that similar meetings may be held in other regions of the world, encouraged perhaps by some of the participants in this meeting. At some time in the near future, once some of the formidable technical problems have been solved, it may be appropriate for some agency such as Unesco to arrange for the exchange of information in this field at an international level.

CHARLES HEIDELBERGER

HAZARDS OF RADIATION

THE committee on radiological hazards to patients, with Lord Adrian as chairman, has now published its second report*. The first, an interim report dealing only with mass miniature radiography, was issued in 1956 and has been reviewed previously (*Nature*, 178, 453; 1956). The present report is devoted to considerations of the long-term genetic hazard to the population as a whole arising from the average radiation-dose received by the population in excess of that from natural sources. The statistical nature of the investigation and of its results is stressed; although doubling the average dose of 3 r. to the gonads during reproductive life might give reason for concern from a genetical point of view, a gonad-dose of 6 r. received by a single individual is not likely to cause abnormalities in the immediate progeny.

The conclusions of the committee are based on two nation-wide surveys. During one week in May 1957, a survey was made in Britain of the numbers and types of X-ray examinations carried out, of the number of films used, and of the apparatus available in all National Health Service hospitals and chest clinics, in dental surgeries and in a few other institutions. Including fluoroscopic examinations, this survey covered a total of 259,457 radiological examinations. The second survey, during one week in December 1957, was made in a random selection of about one quarter of the National Health Service hospitals and chest clinics, and totalled 55,065 radiological examinations. These surveys were supplemented by physical measurements (totalling about 14,000) of the radiation-dose received by patients undergoing different types of radiological procedures in a representative sample of 130 hospitals and chest clinics.

When account is taken of seasonal differences in the frequency of examinations, the data from both surveys agree in indicating a total of 13·0 million radiological examinations during 1957. This is somewhat more than the corresponding figure of 12·2 million used in the Medical Research Council's first report. In large part this may be due to the much more restricted sample that was available for the earlier estimate. More important, and possibly an indication that the recommendations of 1956 had already influenced radiological practice in 1957, was a difference in age distribution of patients examined in the two reports. While in 1956 it had been estimated that 31·9 per cent of all examinations had been given to the age group 0–14, and 25·9 per cent to the over-fifty age group, the corresponding figures in 1957 were 13·5 and 34·4 per cent. From a genetical point of view, this is a desirable development; for the potential effect of a given dose on future generations is greater in a young person having a high expectancy of parenthood than in an older person. In fact, the calculations of genetical hazards in the present report make use of fertility factors giving the average child expectancy at each age for arriving at the "genetic dose to the population". By this is meant the gonad-dose which, if given to every member of the population, would have the same effect as the sum of the individual doses actually received.

The results of the surveys are expressed as mr. (milliroentgens) annual genetic dose per head of the population. All medical radiology in 1957/58, both diagnostic and therapeutic, resulted in a genetic dose of 19·3 mr. This is less than the figure for diagnostic radiology alone (22 mr.) in the Medical Research Council's report of 1956. The main contributions to the total of 19·3 mr. are: diagnostic radiology 14·1 mr., radiotherapy of non-malignant conditions 4·5 mr., radiotherapy of malignant conditions 0·5 mr.; minor contributions are made by medical uses of radioactive isotopes (0·18 mr.), mass miniature radiography (0·01 mr.) and dental radiography (0·01 mr.). The dose to the gonads from any X-ray examination is small in comparison with that considered by the Medical Research Council to be acceptable to the individual without causing undue concern on behalf of himself or his offspring. The committee therefore concludes that there is no need for major restrictions in radiological practice, and that the number and types of examinations or treatments must be dictated by the clinical needs of the patient. Nevertheless, it considers that, whenever possible, the gonad-dose should be reduced even further, and it gives detailed recommendations how this can be done, especially in the case of pregnant women. It is satisfied that, if these recommendations are followed, the total annual genetic dose to the population from all forms of medical radiology can be reduced to a figure of 6 mr. or less. C. AUERBACH

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* Medical Research Council. *The Hazards to Man of Nuclear and Allied Radiations—a Second Report to the Medical Research Council.* (Cmnd. 1225.) Pp. vii+154. (London: H.M. Stationery Office, 1960.) 7*s*.

PHYSIOLOGY OF SUGAR-CANE

R. L. BIELESKI (*Australian J. Biol. Sci.*, 13, 203; 1960) has found that the sugar uptake by sugar-cane storage tissue takes place in two stages. In experiments in which slices of mature and immature storage tissue were used, the initial uptake reached an equilibrium within 1 hr., the level being proportional to the external sugar concentration: it was independent of the sugar, and was unaffected by anaerobic conditions. When the tissue was placed in water, this sugar diffused out rapidly, that is, it was contained in the apparent free space, 10–20 per cent of the tissue volume. The secondary uptake continued up to 60 hr. at a slow, constant rate, 1–5 mgm./gm./day, independent of sugar concentration above 2·0 per cent, dependent on the sugar, and inhibited by anaerobic conditions. This sugar did not diffuse out when the tissue was placed in water. The secondary uptake thus appears to involve an active accumulation process. The sucrose content of the tissue increased during accumulation, which occurred against a 10- to 200-fold concentration gradient. There was no starch synthesis, but accumulation was associated with a 30–40 per cent increase in respiration. Sucrose was not hydrolysed prior to accumulation, and when slices accumulated sugar from a mixed solution, sucrose uptake inhibited glucose uptake. Internodes which were most active in storing sugar in the field gave the most actively accumulating preparations in laboratory experiments. Mature tissue slices showed little or no ability to accumulate sugars.

In further work on the effects of inhibitors on sugar accumulation, the author (*ibid.*, p. 221) has noted that various metabolic inhibitors, at pH 5·5, affect sugar accumulation in immature sugar-cane storage tissues. The rate of accumulation was reduced by 10^{-5} M mercuric ion, 10^{-5} M p-chloromercuribenzoate, cyanide, and cupric ion, and 2×10^{-3} M phloridzin. Accumulation was completely inhibited and sugar leakage induced by 10^{-5} M dinitrophenol, 10^{-4} M mercuric ion, and 10^{-3} M p-chloromercuribenzoate, cyanide, cupric ion, azide, arsenate, and iodoacetate. The effects of 10^{-5} M dinitrophenol and 10^{-4} M cyanide were reversible, but that of 10^{-3} M cyanide was irreversible. Only slight effects were produced by borate, phosphate and magnesium ions. The behaviour of sugar-cane sugar accumulation towards inhibitors is apparently similar to that of other transport mechanisms, being somewhat more sensitive than most plant processes and less so than animal ones. It differed from sugar accumulation in animal tissues in showing no specific sensitivity to phloridzin. The results suggest that sugars are contained within the immature storage cell by the continuous operation of a metabolic storage mechanism rather than by an impenetrable barrier to sugar diffusion.

VIRUSES AND PLANT DISEASES

THE investigations of Dr. L. O. Kunkel into the relationships between viruses and plant diseases are described in the *Rockefeller Institute Quarterly* (4, No. 2; Summer, 1960).

Kunkel discovered that the aster yellows disease is carried by a leafhopper and is caused by one of the few viruses that is equally at home in hosts of the plant and animal worlds. First, he observed that during the hot summer months the aster yellows disease diminishes in plants, and showed that the causative virus is sensitive to heat. Dr. Kunkel was able to cure infected plants of several virus diseases by growing them at temperatures which destroyed the virus without destroying the plants. Peach yellows is one of the diseases which responded to heat treatment. The heat-treatment technique also enabled Dr. Kunkel to deduce that the aster yellows virus multiplies in its leafhopper carrier as well as in the plant it infects. To do this, he subjected the insects to heat and noted how they regained their infectivity. He concluded that the virus must multiply in the insects, reaching a level of maximum infectivity in about two weeks. Direct evidence for this was found by L. M. Black, using a technique developed in Africa by the English scientist, H. H. Storey, to inoculate minute leafhoppers with measured amounts of virus.

Secondly, in his work on the transmission of yellows viruses, Dr. Kunkel discovered two related strains of aster yellows virus, each of which will protect plants against infection by the other, a phenomenon known as cross-protection. He also found cross-protection between viruses in the leafhoppers as well. Dr. Karl Maramorosch, who began work at the Institute with Dr. Kunkel, has continued his studies of plant viruses. He confirmed the complex relationships existing between two strains of the corn stunt virus and the leafhopper that transmits them from plant to plant. Of the two strains of the virus, plants infected by one are protected against infection by the other, but the second will not protect a plant against the first. Similarly, infection of a leafhopper by the first will prevent it from becoming infected afterwards with and transmitting the second. In the insect also, the second virus strain will not protect against the first. These findings regarding corn stunt virus confirmed Kunkel's discovery that two related strains of aster yellows virus would protect leafhoppers against each other, but Dr. Maramorosch was the first to find unilateral protection.

Maramorosch has also found that a leafhopper which normally feeds on corn can live on aster plants only if the asters are infected with the aster yellows virus. After they feed on diseased asters, the corn leafhoppers are then able to survive on uninfected aster plants. They are then also able to survive on healthy carrot plants, which they could not do before their diet of diseased aster plants. It would appear that the virus infection may actually benefit the insect by widening its potential food plants. The explanation of these phenomena is not yet clear, but Dr. Maramorosch is pursuing his investigations.

DISTILLATION OF ALKALI ELEMENTS DURING FORMATION OF AUSTRALITE FLANGES

By DR. S. R. TAYLOR*

Department of Geochemistry, University of Cape Town

THE flanged australites (Figs. 1 and 2) are the most distinctive members of this well-preserved group of tektites. The common australite forms are distinguished by Fenner[1] as round (buttons and lenses), oval (ovals, boats and canoes), dumbbells and teardrops. 'Cores' may be round, oval or dumbbell shapes. The flanges, which occur most commonly on the round forms, but occasionally on the other types as well, display the often quoted evidence for two periods of melting of tektites. Sections

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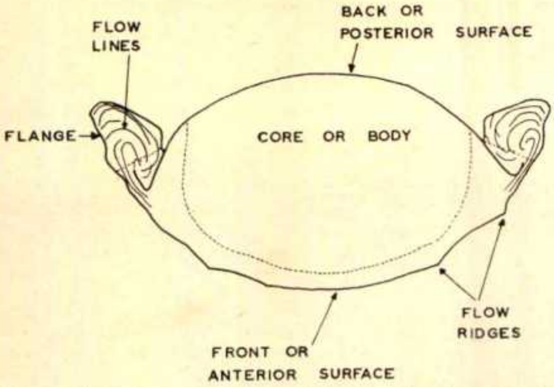

Fig. 1. Typical flanged australite (after Baker[4]). The dashed lines indicate portions analysed

indicate that the material forming the flanges has melted at the anterior surface (Fig. 1) and has flowed back to form the flange around the unmelted core. The flow-lines are clearly shown in the figure by Fenner[2] and in the striking photographs by Fenner[3] and Baker[4,5]. This evidence, together with such features as 'flow ridges' seen on the anterior surfaces, has been interpreted by most workers, as being due to high-speed ('ultra-supersonic') flight through the atmosphere, or to the passage of a blast of hot gas over the tektites. Fenner[3] and Baker[5] have been able to relate the common forms of australites to such a sculpturing process.

The evidence for an origin of the flanges during a second period of melting, although strong, is not universally accepted. Thus Hawkins[6], in discussing a proposed origin of tektites through fusion of sandy clays by lightning, states that: "It is perhaps possible for the buttons with flanges, dumbbells and other shapes to be formed by fusion within the ground. It is not correct to assume that these shapes can only be produced by the melting of a tektite during supersonic passage through the air".

Forms bearing a general resemblance to flanged australites have been made by firing lead bullets against a metal target. Baker[5] shows photographs of these, with well-developed flanges, and a central core.

Very little is known about the relative composition of flanges and cores. Baker[5] has directed attention to the difference in specific gravity between flanges, which average 2·385 and body portions, with an average of 2·426, which suggests a higher silica content for the flanges. Baker and Gaskin[7] give separate analyses labelled australite glass (flange) and australite glass (core). The analyses do not show significant differences, except that calcium oxide is lower in the core. No figures are given for sodium monoxide and potassium monoxide, which are listed as "n.d.".

If the flanges are due to heating and remelting due to passage through the atmosphere, then some selective loss of the more volatile components may be expected. From experience with the behaviour of elements during arcing of silicate powders in spectrographic analysis[8] as well as from data for the boiling points of oxides, losses of such 'volatile' elements as arsenic, mercury, cadmium, bismuth, thallium, indium, silver, zinc, tin, lead, copper, calcium, lithium, sodium, potassium, rubidium and cæsium might be anticipated. Loss of sodium and potassium, which are major constituents, the abundance and distribution of which in australites is reasonably well known, would be simplest to interpret. It is the purpose of this article to show that, in comparison with the core or body, the flanges contain significantly lower amounts of these two elements, and that there are strong indications that lithium, rubidium and cæsium also follow this pattern.

Two imperfect specimens of flanged australites were available as well as a separate piece of flange. These are shown in Fig. 2. The number of specimens, though small, is sufficient to establish whether a significant difference exists between the composition of the core and the flange, and involves a minimum destruction of these interesting objects.

The entire samples were first cleaned by boiling in 5 *N* hydrochloric acid (analytical reagent) and washed in alkali-free distilled water. The projecting portions of the flanges were removed using a small chisel. The remaining portions of the flanges, as well as the anterior surface of the australites, were removed by grinding, leaving the central part of the core for analysis. The portions selected for analysis are shown in Fig. 1. The cleaning treatment was repeated, on the separated cores and flanges, before crushing in a steel mortar to pass 20-mesh nylon cloth, in a plastic sieve holder. A hand magnet was used to extract metal before grinding in an agate mortar to pass 120 mesh.

Sodium and potassium were determined using an EEL flame photometer, under conditions identical with those fully described by Taylor[9]. Natural silicate standards such as the standard granite

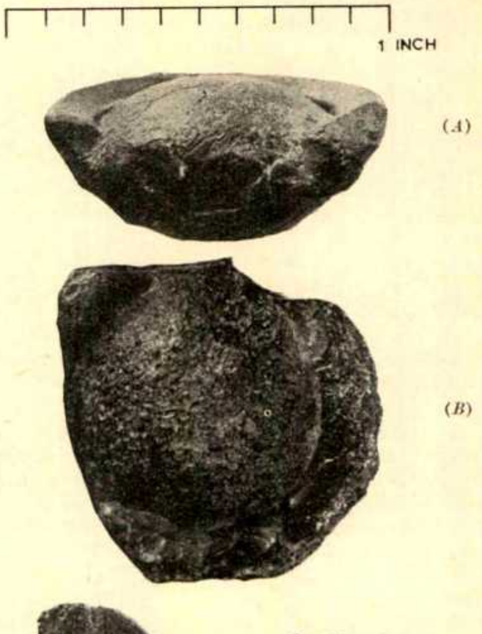

Fig. 2. Flanged australites from (*A*) Charlotte Waters, Central Australia, and (*B*) Mt. Elephant, Victoria. (*C*) Detached flange from Mt. Elephant, Victoria

Table 1. Analytical Data for Cores and Flanges of Australites

Locality	Sample No.		per cent sodium	per cent potassium	p.p.m. lithium	p.p.m. rubidium	p.p.m. cæsium
Charlotte Waters, Central Australia (625)	*A*	core	0·894 ±0·002	1·860 ±0·004	47·4 ±1·8	89·9 ±3·2	2·91 ±0·30
		flange	0·694 ±0·001	1·705 ±0·002	42·4 ±0·8	77·9 ±1·4	2·64 ±0·36
		per cent difference	22·5	8·3	10·5	13·3	9·3
Mount Elephant, Victoria (1)	*B*	core	0·813 ±0·004	1·732 ±0·007	37·3 ±2·5	72·8 ±3·7	1·61 ±0·10
		flange	0·698 ±0·003	1·633 ±0·003	35·7 ±0·8	68·8 ±3·1	1·44 ±0·19
		per cent difference	14·1	5·7	4·3	5·5	10·6
Mount Elephant, Victoria (10)	C	flange	0·635 ±0	1·621 ±0·001	34·5	67·6 ±1·3	1·83 ±0·21
Average of three flanges			0·68	1·65	38	71	2·0
Average values for 24 australites (ref. 9)			0·90	1·85	42	81	2·5
Overall analytical precision (*C*) (per cent)			2·5	2·5	5·8	7·6	14·8

(*G*-1), diabase (*W*-1)[10] U.S. Bureau of Standards soda feldspar (99), plastic clay (98) and the MIT standard biotite (B-3203) have been used and it is believed that systematic error is small.

Lithium, rubidium and cæsium were determined by emission spectrography under conditions similar to those reported by Taylor[9], for the determination of cæsium. Kodak I*L* plates were used in place of I*N*, used previously, and *Li* 8126 was used in place of *Li* 6103. Results are given in Table 1.

It can be seen that for the two samples where data are available for both core and flange, that the flange contains less of the alkali elements than the core. The percentage difference in composition is very much greater than the precision of the individual determinations for sodium and potassium, and these elements must be considered to be significantly lower in the flanges, compared with the cores. For lithium the flange of sample *A* is 10·5 per cent lower than the core; for sample *B*, the difference is 4·0 per cent. Rubidium is 13·3 and 5·8 per cent lower, respectively, in these samples. The difference between core and flange is clear for these elements in sample *A*, and about equal to the precision for sample *B*. The analytical precision for cæsium is much less satisfactory, due principally to the necessity for corrections for background, with a high background to line ratio. The difference in cæsium content between cores and flanges is less than the difference between replicate determinations. The overall precision of the analytical methods expressed as relative deviation, *C*, and based on many determinations, is given in Table 1 as well. It can be seen that the difference between core and flange is less than one relative deviation for lithium in sample *B*, and only about one-half the relative deviation for cæsium in both samples *A* and *B*. In summary, the difference in composition is clear for sample *A* (except for cæsium) and for sodium and potassium in sample *B*, but less clear for the rarer alkali elements in that sample. It is believed that greater precision in the spectrographic determinations would confirm the present indications of loss of these trace elements.

Sample *C* consists of a flange only (the small portion of core which was removed prior to analysis, was insufficient for the determination of sodium and potassium). Only one published australite analysis[11,12] has less potassium (1·04 per cent). No published analysis reports a lower amount of sodium, the minimum figure being 0·68 per cent sodium[12]. For comparison, the averages of the alkali elements in 24 australites[9] are shown. This total includes the two cores given in the same table, but no flanges, and it can be seen that the figures given for the three flanges are substantially lower than these averages.

Fenner[3] and Baker[4,5] discuss the origin of australite flanges by ablation during high-speed flight through the atmosphere, and Baker[5] gives a graphic illustration of this lively process. The loss of the more volatile elements during flange formation indicates that a process such as that envisaged by Baker and Fenner, in fact, occurred. No hypothesis of flange formation by flattening on impact, or by formation at the same time as the initial melting as required by Baldwin, will explain the difference in chemical composition. It is decisive evidence that the flanges are secondary features, due to a second period of melting, during which selective distillation of the alkali elements occurred.

Several points of interest remain for investigation. High-speed flight of tektites through the atmosphere will result if they were formed during the comet explosion, as envisaged by Urey[13], so that the evidence presented here does not rule out a terrestrial origin. The velocity of flight through the atmosphere could be a critical factor. If tektites are of extra-terrestrial origin, they must have entered the atmosphere at velocities in excess of 11·2 km. sec.$^{-1}$. The rather delicate sculpturing of australites, as well as the selective loss of the alkali elements from the flanges, indicate less-severe conditions than those experienced by meteorites. Comparison with the selective distillation of the alkali elements from powdered tektites in a d.c. arc, at temperatures of 3,000–4,000° K. (ref. 8), indicates temperatures of a few thousands, rather than tens or hundreds of thousands of degrees, for the second period of melting of tektites. It is to be hoped that this aspect will be studied by physicists interested in re-entry problems.

In conclusion, it should be noted that the average composition in major elements of tektites may be matched by that of a 3 : 1 mixture of the non-volatile constituents of shale, and quartz[14,15]. The trace element content of australites[16] supports the composition suggested above. It is possible that there is a general deficiency of the more volatile trace elements in tektites, compared with this parent material. Copper[16], gallium[17] and possibly lead seem to be lower (by factors of one-half) than would be expected in such a quartz-shale mixture. Such losses would result from the initial melting process.

The evidence from their chemical composition is strongly in favour of a terrestrial origin for tektites. The external sculpture of australites, and selective loss of alkali elements during flange formation, point unequivocally to a second period of melting. The current enigma presented by the tektites is how to reconcile a terrestrial composition, an initial melting of short duration, and a second period of melting rationally explained by high-speed flight through the atmosphere. Only the comet theory proposed by Urey[13] approaches an adequate explanation.

I am indebted to Miss Maureen Sachs for the analytical determinations. The Director, South Australian Museum, Adelaide, provided sample *A*, and the Director, National Museum of Victoria, Melbourne, sent samples *B* and *C*. Without their generous co-operation, this investigation would not have been possible.

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PALÆOMAGNETISM IN SOME EAST GREENLAND SEDIMENTARY ROCKS

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DURING a visit to Central East Greenland in 1957, on the invitation of Dr. Lauge Koch[1], we collected specimens of sedimentary rocks, with the view of examining their magnetism. This article summarizes the results from the late Pre-Cambrian (two horizons), from the Permian, and from the Middle and Upper Trias. Other rocks collected (igneous rocks, lowest late-Pre-Cambrian horizon, Devonian, Lower Carboniferous, etc.) require further investigation, some being unstable. All the results, with a full discussion, will be published in *Meddelelser om Grønland*, with stratigraphical details and references.

Specimens were oriented in the field by rock compass[2]. In the laboratory the rocks were re-oriented, cored and measured on the astatic magnetometer housed at the Department of Geodesy and Geophysics, Cambridge. For these facilities we are indebted to Mr. B. C. Browne and Dr. J. C. Belshé. Afterwards, a second magnetometer was constructed for this work in the Sedgwick Museum, Cambridge. From each rock one or more samples were measured, and when necessary the vectors combined by taking means of the direction cosines to give a single mean vector for each rock, this mean vector forming the basic unit. After correcting for the effects of dip, the mean vectors from all the rocks of a similar age were combined by taking the mean of the direction cosines, and a statistical estimate of the dispersion was made by Fisher's method. These group mean positions were then used to give the position of the magnetic pole[3]. The main computations were carried out on the Cambridge digital computor *Edsac*.

Table 1

Group	No. of rocks	Dip corrected: *D*	*I*	*α*	*κ*	Position of pole Lat.	Long.	dχ	dψ
Middle and Upper Trias	44	358	68	3	41	160E	68N	5	4
Permian	10	175	−37	7	50	163E	38N	8	5
Tillite Formation	16	135	16	18	5	161W	04N	19	10
Multicoloured Series	10	110	36	9	31	141W	13S	10	5

Explanation of columns:
D, declination in degrees measured from true north.
I, inclination to the horizontal in degrees, positive downwards, negative upwards.
α, semi-angle of the cone of confidence drawn at 95 per cent probability-level.
κ, estimate of precision.
dχ, dψ, semi-axes of the ovals of confidence drawn at the 95 per cent probability-level.

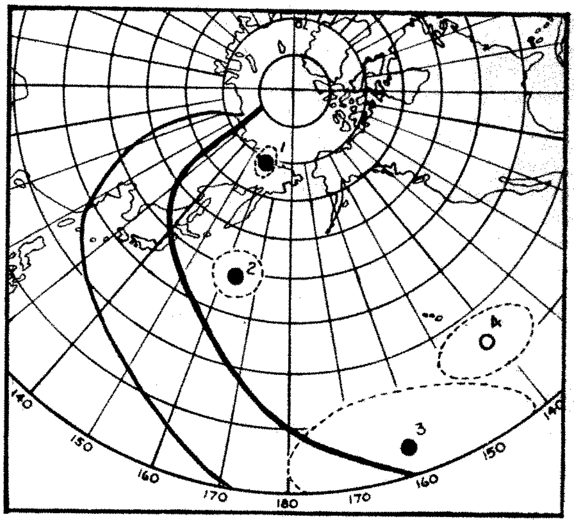

Fig. 1. Polar projection showing positions of magnetic poles derived from (1) Middle and Upper Trias; (2) Permian; (3) Tillite Formation; (4) Multicoloured series. ●, pole plotting in northern hemisphere; ○, pole plotting in southern hemisphere; broken lines show ovals of confidence drawn at the 95 per cent level. For comparison, the approximate path of north pole inferred from British rocks (thick line) and the path inferred from American rocks (thin line) are shown, after Creer *et al.* (ref. 5)

Table 1 lists the group mean positions and pole positions; the latter are shown graphically in Fig. 1.

(1) The Middle and Upper Trias rocks were sampled at Kapp Biot, where 44 specimens of flat-lying red sandstones and marlstones were collected. These were found to be strongly and uniformly magnetized, with eight of the rocks forming a reversed group directed upwards and to the south, whereas the remainder formed a normal group downwards and to the north. The two groups have been combined to give the group mean position.

(2) Ten flat-lying red sandstones of Permian age collected from the two well-separated areas of Gauss Halvö and Mesters Vig were strongly and uniformly magnetized to the south and upwards, forming a reversed group at a shallower angle than the Middle and Upper Trias.

(3) The tillite formation of uppermost Pre-Cambrian age was sampled on both limbs of an anticline on Ella Ø; many of the fifty-four rocks collected proved too weakly magnetized to be measured, or had vectors close to the present field. Sixteen of these rocks have been combined to give the group mean, on the grounds that they show positive evidence of stability of magnetization by the presence of reversals, low scattering of vector positions between rocks from the same collecting station, and by the use of the tectonic check provided by the anticline. The rocks are of varied lithology, ranging from red sandstones and tillites to grey and brown shales.

(4) The multicoloured series (3,500 m. below basal Cambrian) form part of the Eleonore Bay Formation, which is a thick conformable sequence passing upwards through the tillite formation into rocks of Cambrian age. Ten specimens of red mudstone were collected from two outcrops on the south side of Ruth Ø, where the beds dip gently south-east. The vectors fall into a single well-defined group directed downward to the east.

The polar stereographic projection (Fig. 1) shows the positions of the pole computed from the four sets of vectors. These pole positions fall into the same general area of the Pacific Ocean and Asia as the pole positions obtained by other workers for rocks collected in North America and Europe, and the pole positions of the older rocks show the same tendency to approach the equator, apparently crossing it at a time between the tillite formation and the Upper Eleonore Bay Formation. This is in agreement with results for rocks of similar age from Europe[3], and we[4] have already discussed the implications of this equatorial position coinciding with a major glaciation. The pole positions for these Greenland rocks lie generally about 18° east of the general European trend, or about 30° east of the North American trend, which would be consistent with a post-Trias movement of Greenland away from North America.

Taking into account this postulated movement between the continents and the similar movement that has been suggested between Europe and North America[5], the pole positions derived from rocks of similar age from the three areas can be compared. In the post-Cambrian rocks there is some agreement, and if the comparison is extended into the Pre-Cambrian, the pole position derived from the Greenland Upper Eleonore Bay rocks lies intermediate between the pole positions from the Cambrian and Upper Torridonian of Europe, and between the Cambrian and the late Pre-Cambrian positions from the Upper Keweenawan and Beltian of North America, which accords with the stratigraphic relationship of the Upper Eleonore Bay Series to the overlying Cambrian rocks.

We wish to correct an error in quoting E. Irving's work. The second sentence in our recent communication[4] should read "Palæomagnetic studies in Australia suggest that Permian Tillites were near the . . . pole".

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USE OF RADIOACTIVE NITROGEN IN STUDYING NITROGEN FIXATION IN BACTERIAL CELLS AND THEIR EXTRACTS

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THE stable isotope of nitrogen (nitrogen-15) has been successfully employed to follow nitrogen fixation in soil bacteria, blue-green algae and in root nodules of legumes and non-legumes[1,2]. It has also been used recently to demonstrate the incorporation of nitrogen into cell-free extracts of *Clostridium pasteurianum*[3], *Azotobacter vinelandii*[4,5], blue-green algae[6] and those of *Chromatium*[7]. Since the nitrogen-15 technique is time-consuming, some two days being required to complete an experiment from the initial exposure to the gas to the final analysis in a mass spectrometer, we have explored the possibility of using radioactive nitrogen (nitrogen-13, β^+ 1·24 MeV.; half-life 10·05 min.) to study nitrogen fixation in bacterial cells and cell-free extracts prepared from them. We present here details of the technique used together with some of the preliminary results.

Nitrogen-13 has been used previously in chemical[8,9] and biological studies[10]. Carangal and Varner[11], after bombarding thin graphite targets with protons or deuterons in a cyclotron, digested them in acid mixtures and prepared labelled nitrate, nitrite and ammonium compounds which were then supplied to excised plant tissues. Although these workers produced molecular nitrogen-13 by oxidizing labelled ammonia with sodium hypobromite, they did not report its use in biological nitrogen fixation.

In our experiments, nitrogen-13 was produced continuously in the Medical Reseach Council's cyclotron by bombarding coarsely granulated charcoal, retained behind a stainless steel mesh, with

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15-MeV. deuterons. The nitrogen-13 was swept from the target in a stream of pure argon through small-bore nylon tubing to the adjacent experimental laboratory, where it was passed through combustion tubes packed with copper at 750° C. and then through Dreschel vessels containing dilute sulphuric acid and alkaline sulphite to remove the last traces of ammonia and any oxides of nitrogen which might be present. The activity of the gas was continuously monitored and its half-life checked at intervals to confirm its identity. With a deuteron beam current of 40 μamp. and an argon flow-rate of 0·5 litre/min., a nitrogen-13 activity of between 30 and 60 mc./litre was produced, contaminated with about 1 per cent of 1·8 hr. argon-41. For experiments with *Azotobacter vinelandii* (*O* strain) the gas was mixed, with the aid of rotameters, with 10 per cent oxygen, which is essential for nitrogen fixation in this organism.

The biological samples contained in 50-ml. Erlenmeyer flasks were exposed to nitrogen-13 by bleeding off a portion of the gas from the main line. The flasks, suspended from a shaker arm in a water-bath at 30° C., had tightly fitting rubber bungs with inlet and outlet glass tubes for the gas. The inlet tube was drawn out to a fine jet which was just clear of the sample (usually 2 ml.) contained in each flask. Eight flasks were connected in series with sufficient polyvinyl chloride tubing to allow for a thorough reciprocating action (80 oscillations per min.) to effect a good exchange between gas and sample. The rate of flow of the gas into this line of flasks was controlled by a pinch-clip, and could be estimated by observing the rate of bubbling as the gas passed through a Dreschel bottle, containing water, at the end of the line. From this point, the gas rejoined the main line, and was swept into a large polythene container on the roof of the cyclotron building where it was allowed to decay before being discharged into the atmosphere.

Azotobacter vinelandii (*O* strain) was grown for 12 hr. in well-aerated nitrogen-free medium in 3-litre flasks[12]. Cell-free extracts of *Azotobacter* were prepared by disrupting about 0·2 gm. fresh weight harvested cells in 30-ml. aliquots of the culture medium (centrifuged at 144,000*g* to remove whole cells) with an ultrasonic titanium probe (M.S.E. Mullard 20 kc./sec.) for 15 min. as described elsewhere[4,5]. The extracts were then centrifuged for 1 hr. either at 25,000*g* or at 144,000*g* in an ultracentrifuge.

Suitable aliquot parts of the supernatant solutions (containing about 0·2 mgm. nitrogen) left after centrifuging, were put in the Erlenmeyer flasks alternating with boiled control samples and the gas mixtures containing nitrogen-13 were flushed through at a rate of about 0·1 litre/min. The samples were well agitated in the water-bath during this period. After exposure of whole cells or their extracts to the tracer, ordinary nitrogen gas was flushed through for 5–10 min. to remove the dissolved active nitrogen gas from the solutions. The samples were then transferred into plastic counting tubes and assayed in a well-type scintillation counter. Each sample was counted for 30 sec. and the observed counting-rates were corrected for the dead-time of the counter where appropriate, and for decay from a suitable standard time, usually taken as 10–15 min. after the end of the exposure.

Table 1. EFFECT OF COMPOSITION OF GAS ON THE UPTAKE OF NITROGEN-13 BY CELLS OF *Azotobacter vinelandii* (*O* STRAIN)
(Counts per sec. referred to a standard time)

Gas mixtures as percentages		Normal cells	Cells boiled for 15 min. before exposure to tracer	Cells treated with 10^{-3} *M* KCN
Argon containing carrier-free nitrogen-13	Oxygen			
80	20	430	60	44
90	10	450	50	42
95	5	350	52	50
98	2	70	48	46

Activity of gas mixtures, approx. 30 mc./litre; gas flow-rate, approx. 0·1 litre/min.; exposure time to radioactive gas mixtures, 10 min.

Cells flushed with ordinary nitrogen gas for 10 min. after exposure to the tracer. 2 ml. cell suspension containing 0·6 mgm. nitrogen were exposed to the tracer for 10 min. and then either flushed with ordinary nitrogen gas for another 10 min. to remove the dissolved nitrogen-13 or else centrifuged at 3,000*g* and the sedimented cells well washed with 0·05 *M* PO_4^{3-} buffer (*p*H 7·5) before assay.

Table 2. EFFECT OF TIME OF EXPOSURE TO TRACER ON THE UPTAKE OF NITROGEN-13 BY CELLS OF *Azotobacter vinelandii* (*O* STRAIN)
(Counts per sec. referred to standard time)

Time of exposure to the tracer (min.)	Normal cells	Cells boiled for 15 min. before exposure to tracer	Cells treated with 10^{-3} *M* KCN
5	280	40	60
10	540	20	40
20	580	41	40
30	500	64	50

Activity of gas mixture (90 per cent argon–nitrogen-13 and 10 per cent oxygen), approx. 30 mc./litre; gas flow-rate, approx. 0·1 litre/min.

2 ml. of cell suspension containing 0·5 mgm. nitrogen were exposed to the tracer and then flushed with ordinary nitrogen gas for 10 min. to remove the dissolved gas.

The results in Tables 1 and 2 show the effect of gas composition and exposure time respectively on the uptake of nitrogen-13 by whole cells of *Azotobacter*. When the gas mixture contained only 2 per cent oxygen there was a marked falling off in nitrogen-uptake. The optimum incorporation of the tracer was achieved with 10 per cent oxygen, and this mixture was used in further experiments. A substantial uptake of nitrogen by cells was achieved by exposing the cell-suspensions to the gas for 10 min. and this was not increased appreciably by longer exposure times. Similar results were obtained when cell-free extracts were used instead of whole cells.

Since early experiments established that the solubility of the gas was sufficient to result in a substantial radioactive count in the boiled samples, methods were devised to eliminate the dissolved nitrogen. For whole cells, two methods proved effective. In the first procedure cells centrifuged at 3,000 *g* for 10 min. were washed with 0·05 *M* phosphate (*p*H 7·5) and then suspended in the buffer and radioassayed as described previously. In the second method, which also proved effective for cell-free extracts, a suitable gas—nitrogen, oxygen or carbon dioxide—was flushed through the system for 10 min. after the extracts had been exposed to the tracer. Results given in Table 3 show that the degassing procedure was quite effective in removing dissolved nitrogen-13 from cell-free extracts. Later experiments confirmed that passing nitrogen gas through for 5 min. was sufficient to remove all the dissolved nitrogen-13 gas from the extracts.

The incorporation of nitrogen-13 into cell-free extracts prepared by centrifuging disrupted cells for 1 hr. at 25,000*g* or 144,000*g* was similar as shown in Table 3. It is of interest that the hydrogenase activity, determined by hydrogen uptake in a Warburg apparatus with methylene-blue as the acceptor[12], was not detected in particle-free extracts after centrifuging at 144,000*g*. During storage at 4° C.

Table 3. UPTAKE OF NITROGEN-13 BY CELL-FREE EXTRACTS OF *Azotobacter vinelandii* (O STRAIN)
(Counts per sec. referred to a standard time)

Experiment	Normal extracts	Extracts boiled for 15 min. before exposure to nitrogen-13
1. Supernatant solution prepared by centrifuging at 25,000*g*	348	110
2. Supernatant solution prepared by centrifuging at 144,000*g*	311	120
3. Solution as in Exp. 2, flushed with oxygen for 10 min. after exposure to the tracer for 10 min.	400	20
4. Solution as in Exp. 2, flushed with carbon dioxide for 10 min. after exposure to the tracer for 10 min.	250	10
5. Solution as in Exp. 2, flushed with nitrogen for 10 min. after exposure to the tracer for 10 min.	200	10

Activity of gas mixture (90 per cent argon–nitrogen-13 and 10 per cent oxygen), approx. 20 mc./litre; gas flow-rate, approx. 0·1 litre/min. exposure time to radioactive gas mixture, 10 min.

Extracts containing approximately 0·15 mgm. nitrogen were flushed for 10 min. after exposure to the tracer, with either oxygen, carbon dioxide or nitrogen. There is no significant difference between the flushing efficiencies shown by Exps. 3, 4 and 5 compared with no-flushing in Exps. 1 and 2.

the particle-free extracts lost their capacity to fix nitrogen more readily than did those centrifuged at the lower speed. Cell-particles appear to be necessary for sustained incorporation of nitrogen-13. Labelled compounds can be detected in the *Azotobacter* extracts and some of them can be removed from the reaction mixtures into various traps. Work is now in progress to determine whether these compounds are intermediates in the fixation process or whether they are formed during side reactions.

Cultures of *Azotobacter vinelandii* (*O*) were kindly supplied by Dr. P. W. Wilson and Dr. R. H. Burris, Wisconsin University, Madison, U.S.A. Mr. G. R. Newbury, formerly with the Medical Research Council Radiotherapeutic Research Unit, first prepared nitrogen-13 by the technique described here. We acknowledge with thanks the valuable assistance of Mr. D. D. Vonberg and the cyclotron team, and the interest and encouragement of Dr. C. A. P. Wood, director of the Medical Research Council Radiotherapeutic Research Unit, and of Prof. H. G. H. Kearns, director of the University of Bristol Research Station, Long Ashton. We also thank Messrs. David Fisher, M. A. Wright, C. A. Fewson and M. A. Proctor, who assisted with certain aspects of the biochemical work, and Messrs. Buckingham and Forse, who assisted with the gas-processing apparatus.

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GROWTH OF BACTERIA LABELLED WITH HEAVY ISOTOPES FOR THE ISOLATION OF NUCLEIC ACIDS

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IN studying the interactions of nucleic acids, as well as their genetic fate and function using the technique of cæsium chloride density gradient centrifugation[1], it is desirable to prepare deoxyribonucleic acid (DNA) as well as ribonucleic acid (RNA) of high density by homogeneous labelling. Both ^{15}N and $^{13}C^{15}N$ have been used successfully for this purpose[2,3]. Unfortunately, the substitution of 5-bromouracil for the thymine in the DNA of bacteriophages, bacteria and tissue culture often results in an inhomogenous distribution of label in the DNA, causing it to form bands over a broad range of density[4].

Conditions have been ascertained that will allow organisms with simple growth requirements to grow on 99+ per cent heavy water (D_2O) containing $^{15}NH_4Cl$ as the sole source of nitrogen. More fastidious organisms (for example, *Diplococcus pneumoniae*) can be grown on a complex medium generally used for the organism, replacing the water with heavy water.

The D_2O-^{15}N medium employed is a modification of that used by G. S. Stent (personal communication) and consists of the following:

Table 1

KCl, 150 mgm.
$^{15}NH_4Cl$*, 25 mgm.
NaCl, 500 mgm
K_2HPO_4, 100 mgm.
Na_2SO_4, 5 mgm.
$MgCl_2.6H_2O$, 10 mgm.
$CaCl_2$, 0·2 mgm.
$FeCl_3.6H_2O$, 0·2 mgm.
Glycerol, 0·15 ml.
60 per cent sodium lactate, 0·02 ml.
D_2O† to make 100 ml.

* Obtained as 99 per cent excess nitrogen-15 from Isomet Corporation, Palisades Park, N.J.

† D_2O of 99·7 per cent purity was obtained from Liquid Carbonic Co., California.

Phenol red indicator is added, the *p*H is adjusted to 7 with NaOD, and the solution is sterilized by filtration. The addition of a small amount (20 μgm./ml.) of casein hydrolysate stimulates growth in heavy water if $^{15}NH_4Cl$ is to be omitted.

Since the bacteria seem to take up water preferentially in the initial stages of growth, resulting in a bimodal distribution of DNA density, the following steps have been taken to avoid traces of water in the 99 per cent D_2O medium. The mixture (omitting the trace elements) is first dissolved in 10–25 ml. of heavy water and evaporated to 1 ml. Then heavy water and trace elements are finally added to make 100 ml. Stock solutions should be prepared using heavy water as the solvent. Solutions should not be autoclaved, because the medium will exchange with ordinary water to the extent of several per cent.

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Most organisms will grow very slowly, and some not at all, if suddenly exposed to high concentrations of heavy water. To overcome this difficulty, growth is initiated by inoculating about 10^5 cells into a small aliquot (about 1 ml.) of the heavy water medium to which a small quantity of sterile physiological saline (in ordinary water) has been added, so that the concentration of D_2O is about 60–70 per cent. The cells are exposed to the normal conditions of temperature and aeration and when the cell titre reaches about 10^8 per ml., fresh aliquots of the 99 per cent heavy water medium are added periodically to replenish nutrients and neutralize the culture medium. Neutralization in the latter stages of growth is best accomplished by adding (with concomitant swirling) the same medium adjusted to *p*H 10 or 11. In some cases (for example, *Bacillus subtilis*) this gradual adjustment of *p*H is preferred, since, if the alkalinity accidentally reaches too high a level, cell lysis will occur. Once the cells have been adapted to growing in 99+ per cent heavy water, they may be used in subsequent harvests by inoculating directly into fully deuterated medium. Since the adaption of micro-organisms to heavy water is slow[5], cultures should be checked periodically for contamination.

The D_2O-^{15}N medium has been employed successfully for the growth of *Alcaligenes faecalis, Salmonella typhimurium, Pseudomonas aeruginosa, Aerobacter aerogenes, Klebsiella pneumoniae, B. cereus, B. subtilis, Escherichia coli* and *Serratia marcescens. D. pneumoniae* was grown in a complex growth medium containing 99 per cent heavy water. Viable titres of 5×10^8 bacteria/ml. have been attained with most of these bacteria. DNA was isolated from the cells by the method of Marmur (unpublished work) in the presence of ordinary water containing reagents. The deuterated nitrogen-15 containing DNA is of high molecular weight (about 10×10^6 as estimated from the band-width in the cæsium chloride gradient). In the case of *Serratia marcescens* DNA, it was found that the mid-point of the rise in absorbancy at 260 mμ as a function of temperature was the same for the labelled and unlabelled samples in ordinary physiological saline, indicating that strand separation takes place at the same temperature for both. DNA isolated from wild-type *B. subtilis* grown in this medium exhibited the same activity in biological transformation as similar non-labelled DNA. The buoyant densities in cæsium chloride of the DNA samples isolated thus far are listed in Table 2.

It should be noted that the heavy DNA obtained showed a typically narrow distribution of bands in a cæsium chloride density gradient when examined in the analytical ultracentrifuge. The variances of the heavy labelled and normal DNA bands agreed within 20 per cent. This seems to be a considerable improvement over the 5-bromodeoxyuridine labelling technique, which usually results in heavy-labelled material producing a band skewed in the direction of light density. In addition, with the deuteration technique, no visible amount of the non-labelled DNA or bands of intermediate density are found. Fractionation by cæsium chloride preparative density gradient, which has been reported in the work with 5-bromouracil containing DNA, is therefore unnecessary with deuterated DNA preparations.

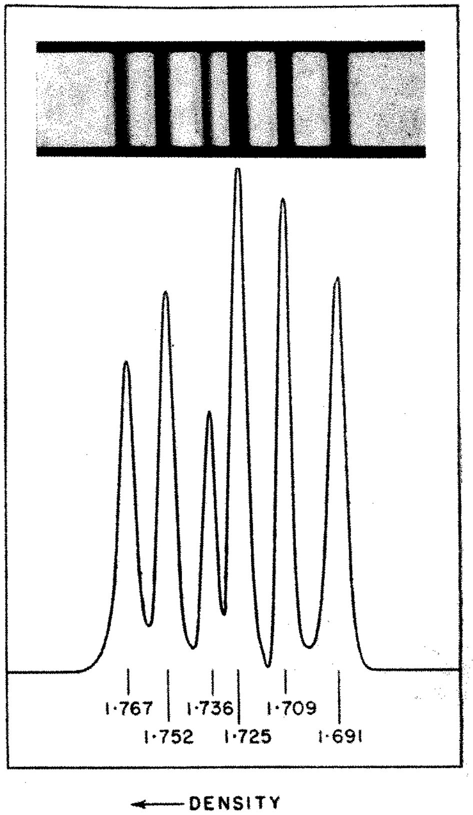

Fig. 1. Ultra-violet absorption photograph and microdensitometer tracing of normal and various labelled samples of DNA isolated from *E. coli* B. Bands have been made in a cæsium chloride density gradient formed by centrifugation at 44,770 r.p.m. The photograph is taken after 20 hr., at which time equilibrium has been established. In order of increasing density, there is DNA from *Clostridium perfringens* (often used as a standard in such work) and different samples of *E. coli* DNA. The first is normal *E. coli* DNA, followed by nitrogen-15-labelled; deuterated; both nitrogen-15 labelled and deuterated; and finally quickly cooled, nitrogen-15 labelled, deuterated DNA

Fig. 1 shows a photograph and tracing of DNA bands in a cæsium chloride density gradient. These illustrate some of the samples labelled with heavy isotopes that have been prepared from *E. coli*. In the deuterated DNA, all deuterium bound to carbon should not exchange with the hydrogen of the water of the physiological saline in which the DNA is isolated. The buoyant density of the DNA in cæsium chloride should thus remain exactly the same after a few months in this solution and even after extensive exposure to temperatures up to 100° C. This has, in fact, been found, eliminating the possibility that slow exchange may take place after isolation of DNA. The quickly cooled, thermally denatured DNA is 0·015 gm./c.c. heavier than the native ^{15}N-deuterated sample. This is the same increase as has been reported for unlabelled DNA (ref. 6).

Table 3 lists the molecular weights of some of the monomer units in the cæsium salt of normal DNA and that labelled with heavy isotope. The densities calculated from the percentage increase in molecular weight due to labelling *E. coli* DNA are 1·724 gm./cm.^{3} for nitrogen-15 substitution and 1·745 gm./cm.^{3} for both nitrogen-15 and deuterium substi-

Table 2. BUOYANT DENSITY OF LABELLED AND UNLABELLED DNA IN CÆSIUM CHLORIDE

Organism	Normal	DNA density (gm./c.c.)		
		^{15}N labelled	D labelled	^{15}ND labelled
**B. cereus*	1·696			1·714
D. pneumoniae	1·700		1·719	
B. subtilis	1·703			1·751
E. coli B.	1·709	1·725	1·736	1·752
* *S. typhimurium*	1·710			1·750
**A. faecalis*	1·715			1·756
**K. pneumoniae*	1·716			1·751
**A. aerogenes*	1·717			1·756
S. marcescens	1·717	1·733		1·758
**P. aeruginosa*	1·726	1·742		1·764

* Cells grown in the medium containing 90–95 per cent D_2O plus $^{15}NH_4Cl$ and the density of the heavy DNA determined directly on a whole-cell lysate. Determinations on other samples were on purified DNA preparations.

Table 3. MOLECULAR WEIGHTS OF MONOMER UNITS OF DNA LABELLED WITH HEAVY ISOTOPE

	Adenine	Thymine	Guanine	Cytosine
Base	134	125	150	110
^{15}N base	139	127	155	113
Deuterated base	138	130	154	114
Deuterated base—exchangeable deuteriums	136	129	151	112
Deoxyribose phosphate	178	178	178	178
Deuterated deoxyribose phosphate	185	185	185	185
^{15}N nucleotide (cæsium salt)	450	438	466	424
Deuterated nucleotide—exchangeable deuteriums (cæsium salt)	454	447	469	430

tution. Since hydrogens bound to oxygen or nitrogen exchange rapidly with water during the isolation of DNA from the bacteria grown in the heavy water medium, the exchangeable hydrogens are not included in the increase of density. It is also assumed that there is no change in volume of the molecule on substitution of the heavy isotope. The predicted density shift for substitution of nitrogen-15 has been observed for the three samples listed in Table 2. Thus far, using the glycerol-lactate medium, it has not been possible to obtain 100 per cent deuterated DNA from *E. coli*. Work is now in progress with a medium containing completely deuterated acetate as the only source of carbon, in order to see whether the DNA isolated will have the theoretical maximum density.

The heavy-labelled DNA prepared by the procedure outlined here has been used extensively in physical chemical studies of strand separation and recombination and in the formation of 'hybrid' DNA molecules. Results of this work will be reported soon by C. L. Schildkraut, J. Marmur and P. Doty. The procedure described can, of course, be adapted for other purposes, such as preparing 'heavy' whole cells, phage, proteins, RNA and other cell constituents.

We are indebted to Prof. Paul Doty for numerous helpful discussions and constant encouragement. The advice and aid of Dr. D. M. Green are gratefully acknowledged. This investigation was supported by a research grant (*C*-2170) from the National Cancer Institute, National Institutes of Health.

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NATURE OF THE RIBOSOMES PRESENT IN BACTERIA AND YEAST: A RE-APPRAISAL

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RIBONUCLEOPROTEIN particles ('ribosomes') appear to form a major constituent of bacterial cytoplasm, and the early work of Schachman, Pardee and Stanier[1], using the ultracentrifuge, showed that ribosomes were responsible for two fast-moving boundaries (29*S* and 40*S*) seen in schlieren diagrams. Tissières and Watson[2] worked with isolated preparations and found the sedimentation coefficients of these particles to be 32*S* and 51*S* respectively when corrected to 20° C. and zero concentration, and we shall refer to them as the '30*S* (or 50*S*) ribosomes'. Ribosomes are also present in animal and plant tissues[3] and in yeast[4], and are generally believed to be a site of protein synthesis[5]; in animal tissues they are often attached to membranes, but in bacteria they appear free in the cytoplasm.

Analytical ultracentrifugation shows that ribosomes isolated from plant and animal sources are larger than the 50*S* ribosomes of bacteria, although the latter can be made to combine with equal numbers of 30*S* ribosomes to form particles sedimenting at 70*S* when the concentration of magnesium ions is raised and phosphate ions are excluded[2]. Since ribosomes isolated from other sources sediment at 70–80*S*, it seems to be generally assumed that inside bacteria there normally exist particles of this size with specific biochemical functions, and that they are liable to dissociate unless special precautions are taken. Accordingly, when cell-free extracts are prepared from disrupted bacteria for ultracentrifuge examination, it is usually the practice to extract with 2-amino-2-hydroxymethyl propane-1 : 3-diol (*tris*) buffer containing magnesium acetate to 'stabilize' the ribosomes. The fact is often ignored that 70*S* ribosomes may be produced from smaller ones by the method of extraction: originally they may not have been present inside the intact cells.

Three fundamental questions concerning ribosomes require attention. (1) Is the ribonucleoprotein of the living cell present as particles ? (2) If so, what are the sizes of bacterial ribosomes ? (3) Is some ribosomal function dependent on size, so that a 70*S* ribosome plays a vital part in metabolism for which smaller ribosomes cannot be substituted ? As regards

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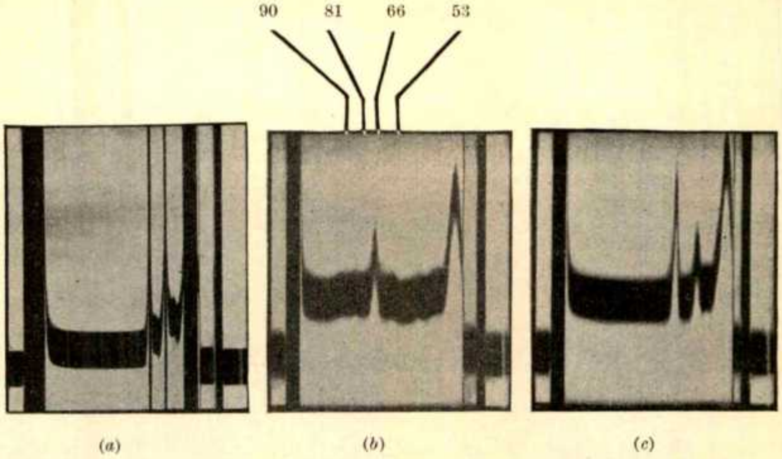
(3), it has been shown[6] that the sizes of ribosomes in bacterial extracts are greatly affected by concentrations of certain salts in the media from which the cells are harvested prior to disruption. Intracellular concentrations of normally metabolizing bacteria do not appear to be so regulated as to preserve intact the 70*S* ribosome; accordingly, particles of this size would not be essential for normal metabolism, although they may well exist when intracellular conditions are favourable. As regards (1), Swedish electron microscopists[7] have produced evidence that all the particles may be formed by precipitation of ribonucleoprotein during the preparation of materials for examination. Certainly, if the ribonucleoprotein in the living cell were not 'rolled up' into particles, the RNA might be accessible and its function as a template in protein synthesis would be easier to visualize; but any possible connexion between particle size and metabolic function would remain obscure. The present investigations were undertaken to answer question (2).



(*a*) (*b*) (*c*)

Fig. 2. Schlieren diagrams: sedimentation to the left; centrifugal field 187,000*g*. (*a*) Protein concentration, 6 per cent. Sedimentation coefficients (uncorrected) of leading boundaries, 21*S* and 14·5*S* respectively; these are due to 50*S* and 30*S* ribosomes. Photographed at 16 min. (*b*) Protein concentration 1 per cent in *tris* buffer containing 10^{-2} *M* magnesium acetate. The numbers above the photograph (at 8 min.) give uncorrected sedimentation coefficients in Svedberg units. (*c*) Protein concentration 1 per cent in phosphate buffer ($1{\cdot}2 \times 10^{-2}$ *M*). Leading boundaries, due to the 50*S* and 30*S* ribosomes, sediment at 43*S* and 27*S*. Photographed at 8 min.

Determinations of ribosomal size are beset with two difficulties: first, the cells are usually disrupted in a medium that may cause immediate dissociation or aggregation; and second, the extract must be further diluted so as to be suitable for ultracentrifugal analysis. We have employed the bacterial press of Hughes[8], in which almost dry pastes may be disrupted, and have examined the whole crush, including debris, in a cell of light-path of only 3 mm. which permits examination at a dilution of 1 part by weight of crushed cells to 1 part or less of water or buffer. The viscosity of such an extract, however, is so great that a particle cannot be identified from a single sedimentation coefficient: thus the 50*S* ribosome may sediment at about 20*S*. We therefore determined sedimentation coefficients for the faster boundaries at a series of dilutions and plotted them against the protein concentrations of extracts (Fig. 1). The leading boundaries in Fig. 2*a* could then be shown to be due to the 50*S* and 30*S* ribosomes, since these sedimentation coefficients were given by extrapolation to zero concentration. No boundary due to a ribosome sedimenting faster than this is seen in Fig. 2*a*, and this result was also obtained when other concentrated crushes of *Esch. coli* and *B. cereus* were examined. In two experiments a very small peak, possibly due to a 70*S* ribosome, was seen. Phosphate buffer ($1{\cdot}2 \times 10^{-2}$ *M*, *p*H 7) was used to prepare extracts in most cases, but similar results were obtained using water.

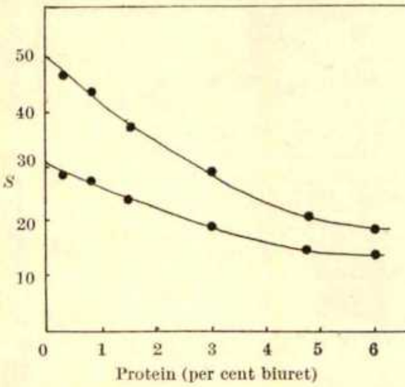


Fig. 1. Sedimentation coefficients (uncorrected) of the larger ribosomes in suspensions of disrupted cells of *Esch. coli*. Rates of sedimentation were measured at a series of dilutions in phosphate buffer ($1{\cdot}2 \times 10^{-2}$ *M*, *p*H 7)

When disrupted bacteria were extracted with 10^{-2} *M tris* buffer containing 10^{-2} *M* magnesium acetate, several larger species were present. A schlieren diagram (12 mm. cell light-path) for such an extract, diluted to the protein concentration (1 per cent) which it has hitherto been customary to use, is shown in Fig. 2*b*. It may be contrasted with that for an extract prepared in dilute phosphate buffer containing no magnesium acetate (Fig. 2*c*). This shows the same main features as Fig. 2*a*, where cell constituents were examined at concentrations much closer to those inside living cells. It appears that the practice of adding a high concentration of magnesium acetate (10^{-2} *M*) to all cell-free extracts before examination, in order to 'stabilize' large ribosomes, is arbitrary and may be misleading.

A similar series of experiments has been conducted with *S. cerevisiae*, and in this case it was possible to examine the juice from crushed cells in the 3-mm. cell without further addition of liquid. From dilution experiments it emerged that the principal (leading) boundary was due to an 80*S* ribosome, as reported for dilute extracts by Chao and Schachman[4]. It therefore appears that the main ribosome (50*S*) normally present in the strains of *Esch. coli* and *B. cereus* that we have studied is of different size from that of yeast (80*S*) or mammals.

Addendum. Very recent work[9] shows that particles from *Esch. coli* sedimenting at 70*S* incorporate amino-acids. In amount, they constitute less than 10 per cent of the total ribosomes and differ from 70*S* ribosomes in so far as they do not appear to be formed from 50*S* and 30*S* ribosomes and are not sensitive to changes in concentrations of magnesium ions. It is therefore more readily understood why the metabolism of bacteria remains unaffected by salt concentrations that can cause profound changes in the distribution of their ribosomes[6]. However, the function of most of the ribonucleoprotein responsible for ultracentrifuge schlieren diagrams of crude extracts remains obscure.

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A PLAQUE METHOD FOR ASSAYING SOME VIRUSES ISOLATED FROM COMMON COLDS

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WE have recently described the isolation of some hitherto uncultivable viruses from human beings suffering from common colds[1-3]. Many more strains have now been isolated[4]. In order to study the properties of a few of these in some detail a simple and precise method of assay was required and methods based on counting plaques have obvious advantages. This article reports the development and testing of such a technique.

In preliminary experiments we attempted to produce plaques by methods closely resembling those originally described by Dulbecco[5]. However, a marked cytopathic effect did not appear in stationary cultures, and the cell sheet did not survive under agar with the roller tubes and media used. An attempt was therefore made to count the focal lesions (Fig. 1) described earlier as the first stage in the cytopathic effect of viruses of this type[3]. This has previously been done with herpes virus[6], vaccinia virus[7] and using enteroviruses in tube cultures[8]. The foci or plaques were counted over the whole cell sheet in every tube using a total magnification of 30–40 ×. Countable plaques were observed with two strains of virus (*P.K.* and *H.G.P.*) which produce a cytopathic effect in monkey kidney cells and in human amnion cultures. Plaques were also counted with viruses growing in human kidney cells; the plaques were, however, more difficult to count because the cell sheet was less uniform than with monkey kidney cells. For practical reasons nearly all the remaining experiments were performed with *H.G.P.* virus in cultures of monkey kidney cells.

The tissue cultures used were secondary cultures of rhesus kidney cells. Between 30,000 and 40,000 cells were inoculated into stationary tubes in 0·5 ml. of a medium consisting of 5 per cent calf serum and 0·5 per cent lactalbumin hydrolysate in Hanks's saline containing 0·03 per cent sodium bicarbonate and 100 units of penicillin, 100 γ of streptomycin and 20 units of mycostatin per ml. Cultures were incubated at 36° C., usually for 12–24 hr. until ready for use. Inoculation took place after the medium had been changed to 1·5 ml. of one containing 2 per cent calf serum and 0·25 per cent lactalbumin hydrolysate, the other constituents remaining the same. After inoculation cultures were incubated at 33° C. in a roller drum. The virus strains had been isolated as described elsewhere[1-4]; *p*H determinations were made with a glass electrode instrument. The plaque count was only calculated from groups of cultures in which every tube had a countable number of plaques or no plaques. Ten-fold or 3·2-fold dilutions were usually employed so that it was possible to combine the results of several dilutions, 3–5 tubes being inoculated with 0·3 ml. of each dilution.

Statistical Aspects of Microplaque Counts

The number of plaques counted by two observers usually agreed quite closely, but there were difficulties at times because of lack of experience in searching the cell sheet systematically. Other discrepancies were reduced when it was agreed (*a*) that a focus must contain about 10 cells to be scored as a plaque, (*b*) that counts of more than 15–20 plaques be excluded. The exact number depends on the degree of compact-

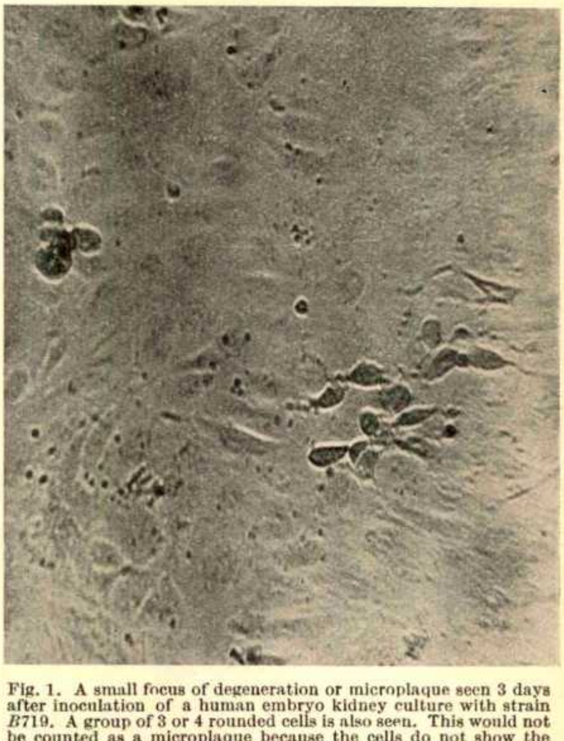

Fig. 1. A small focus of degeneration or microplaque seen 3 days after inoculation of a human embryo kidney culture with strain *B*719. A group of 3 or 4 rounded cells is also seen. This would not be counted as a microplaque because the cells do not show the typical changes induced by the virus. (Unstained × 240)

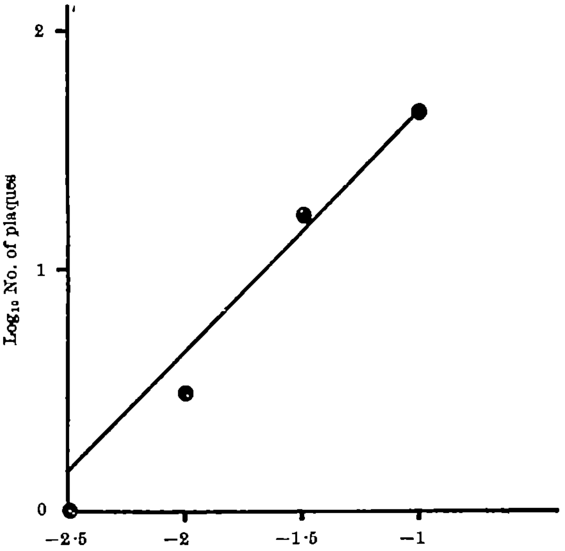

Fig. 2. The relationship between plaque count and the concentration of virus in the inoculum

ness of the plaques, which differs between batches of monkey kidney cells.

In numerous experiments it was shown that until three days the number of plaques occurring was directly proportional to the concentration of virus inoculated. An experiment of this type is shown in Fig. 2. In a χ^2 test applied to the original data the deviations from expectation were found not to be significant.

The distribution of plaque counts was compared with that to be expected on the basis of a Poisson distribution. Fifty tubes were inoculated with a pool of virus diluted 1/100. As can be seen from Fig. 3, the number of plaques at three time-intervals after inoculation agreed well with the distribution calculated from the mean number of plaques per tube. The deviations from expectation were tested by the χ^2 test, and in each case P was greater than 0·1. After three days the number of plaques increases in all tubes near the end-point while negative tubes remain negative We assume that 2–3 days following inoculation an increasing proportion of the virus particles which have become attached to the cells are able to produce visible plaques, and that later plaques are secondary to the earlier plaques. In some experiments the plaque count at three days was compared with the titre of virus as judged by the 50 per cent end-point method used before[3]. The highest plaque count obtained before the distribution became non-Poissonian seemed to be equivalent to the 50 per cent infectivity end-point method.

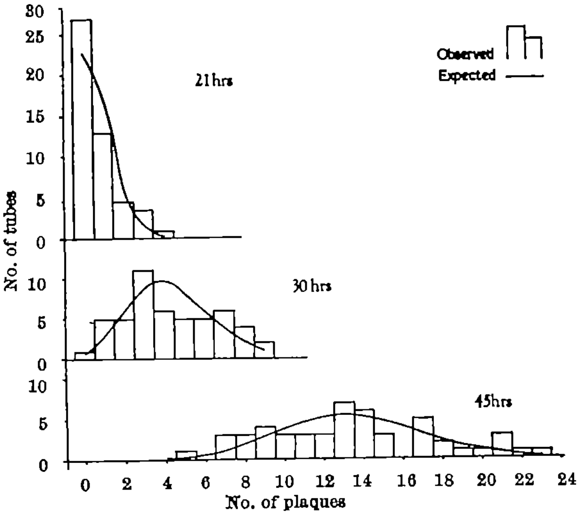

Fig. 3. The number of microplaques counted compared to those expected according to the Poisson distribution

It was shown that if *H.G.P.* virus was mixed before inoculation with a specific hyperimmune antiserum (prepared in a rabbit) the plaque count was reduced· These experiments will be reported in full later.

Optimal Conditions for the Production of Microplaques

(*a*) *Temperature and pH of medium.* It was discovered that the conditions previously found necessary for the production of a general cytopathic effect were also necessary for the appearance of the maximal number of microplaques. With a strain of *H.G.P.* well adapted to monkey kidney cultures, the temperature was found to affect the plaque count, as can be seen from Table 1. Similar results were obtained when virus-infected nasal washings were used as the inoculum.

Table 1. Effect of Temperature of Incubation on the Number of Plaques Developing

Inoculum	Day of reading	Plaque count on cultures incubated at		
		30° C.	33° C.	36° C.
Virus passed 5 times in human embryo kidney and 8 times in monkey kidney cells	Day 2	12	1,200	190
	Day 3	1,400	14,000	150

We wished to know if changes in bicarbonate concentration were important in themselves or because of changes they induced in the *p*H of the medium. Titrations were therefore set up in parallel cultures maintained in media containing various concentrations of bicarbonate as buffer, or else sodium phosphate buffer. The number of plaques appearing was counted and the *p*H of the medium in each case was estimated. The *p*H usually dropped about 0·3 unit between the beginning of the experiment and the time of reading, but the change was greater in cultures buffered with bicarbonate and less in tubes buffered with 0·05 *M* phosphate. The results of two such experiments are shown in Fig. 4. It seems that the *p*H rather than the concentration of bicarbonate affects the plaque count and that optimal conditions were obtained when the medium had a mean *p*H of 7·0–7·4. The *p*H of the medium at the end of the experiments showing optimal results ranged from 6·9 to 7·1. This was achieved by adding phosphate buffer *p*H 7·14–7·69 at a final concentration of 0·05 *M* to a medium without bicarbonate.

(*b*) *Volume of inoculum.* In several experiments, stationary cultures at 33° C. were overlaid with a standard dose of virus contained in various volumes of medium. 4 hr. were allowed for adsorption of virus ; after this the inoculum was poured off and the cultures were washed before fresh medium was added and the tubes were rolled (Table 2). It can be seen that high titres were obtained with rather small volumes of fluid but the titres were no higher than those obtained when we used 1·5 ml. and rolled the cultures immediately following inoculation. The

Table 2. Effect of Inoculum Size on the Plaque Count

Size of inoculum	0·1	0·3	0·6	0·9	1·5 ml.
Plaque-forming units/ml.	1,200	1,200	1,100	840	1,300

reason for this is not understood as we expected the virus to become attached to the cells most successfully when small volumes were used ; possibly even a short period under stationary conditions reduces the susceptibility of the cells to virus.

(*c*) *Time of exposure to inoculum*. Cultures were inoculated with dilutions of virus and incubated under standard conditions. At intervals the medium was poured off sets of culture tubes (donor tubes) and inoculated to other sets, to ascertain how much virus had disappeared from the medium. In some experiments the donor tubes were washed or donor tubes were used which contained no cells. Some typical results are summarized in Fig. 5. This indicates that 80–90 per cent of virus added to the tubes is rapidly adsorbed. However, the virus has to be left in contact with the cells for 2–4 hr. in order to give maximal plaque counts. It is probable that much of the virus which disappears from donor tubes is adsorbed to the glass of the tubes since virus disappears from dummy donor tubes which contain no cells.

Discussion

Since the experiments described above were performed it has been found that the microplaque method can be readily applied to the measurement of relative virus concentration for various purposes. It has also been used to measure quantitatively the neutralizing activity of sera and nasal secretions, and to assay the effect of interferon on monkey kidney cells[9]. We are also using it to assess the effect of adverse conditions such as high temperature and pH on different phases of the growth-cycle. The main difficulty of the test is the necessity to read the cultures at what often prove to be awkward times, since if cultures with, say, 1 or 2 plaques are left for 24 hr. they may be unreadable because the plaques are confluent. In addition, with ill-adapted viruses or with uneven batches of tissue cultures the results may be less satisfactory than those reported here. Nevertheless, since using this technique we have been able to accumulate, with the use of relatively small amounts of tissue culture cells, more precise data than was previously possible.

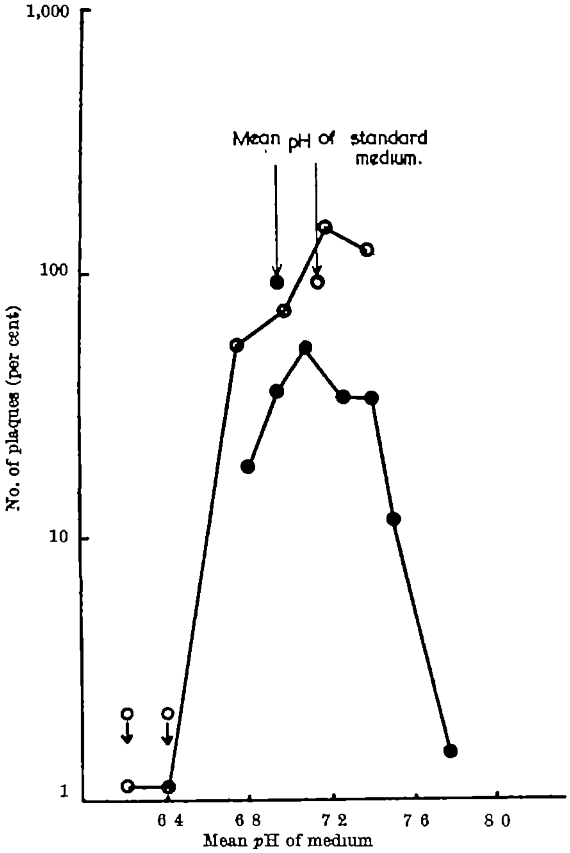

Fig. 4. The number of plaques developing in tissue cultures containing media buffered with phosphate at various pH's—expressed as a percentage of the number of plaques in cultures containing the standard amount of sodium bicarbonate (0·03 per cent). ○, exp. 1 ; ●, exp. 2.

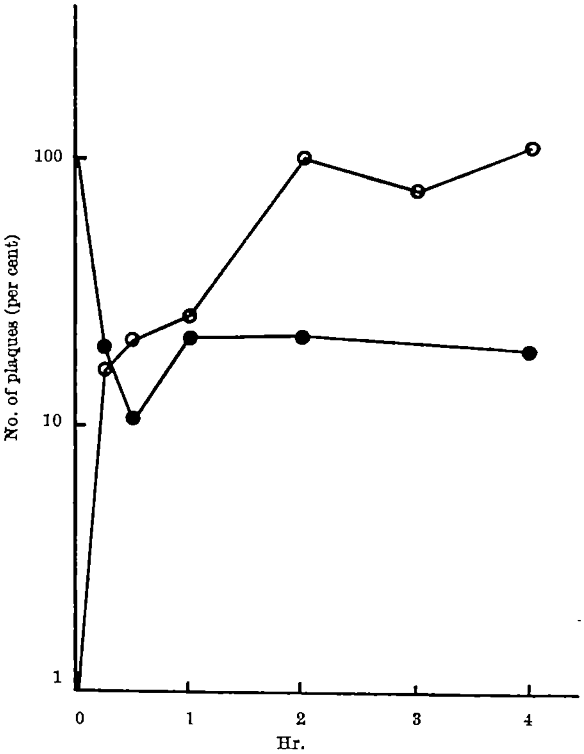

Fig. 5. ○, Number of plaques appearing in a series of dilutions when the inoculum was removed at various times, as percentage of plaques found when the inoculum was not removed , ●, residual virus recovered from the medium of dilution tubes, assayed by transfer to fresh tissue cultures, and expressed as percentage of plaques found when the inoculum was not removed. The amount of virus left in the medium was proportional to the concentration of virus inoculated

We wish to thank Mr. K. Allner for the photomicrograph and Mrs. P. A. Palmer for technical assistance.

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FLUORESCENCE AND HÆMOLYTIC ACTIVITY OF TUMOURS AND NORMAL TISSUES OF THE *C3H* MOUSE

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LYSINS have been extracted from normal mouse tissue, from mammary carcinomas of female *C3H* mice[1], and from lymphomatous glands of *AKR* mice[2]. They have been partially identified as C-18 fatty acids[3], such as oleic, ricinoleic or related fatty acids, for example, the cytolysin found in the royal jelly of bees, 10-hydroxydecenoic acid, lethal for leucæmic and ascitic tumour cells[4], the antibiotic C-18 fatty acid found in royal jelly (also 10-hydroxydecenoic acid)[5], and the as yet unidentified fatty acid in the microsome fraction of guinea pig liver cells[6,7].

This communication is concerned with measurements of the amount of fatty acids found in the normal tissues and the tumours of *C3H* mice, using fluorescence methods and hæmolytic titrations.

Mammary tumours are dissected from the white tumours of *C3H* mice after the animals have been bled by cardiac puncture. About half the material, minced with scissors, is weighed, dried to constant weight at 60° C. and re-weighed. This gives the dry material of the tumour.

The remaining half of the wet mince is transferred to the thimble of a micro-Soxhlet apparatus and is extracted for 2 hr. with 25 ml. of hot benzene which is made up to 50 ml.; this gives the unknown X which contains the C-18 fatty acids and other substances extracted by hot benzene from a known weight of wet tumour mince.

A standard $M/100$ solution ($= 2{\cdot}8$ mgm./ml.) of ricinoleic or oleic acid in 50 ml. of benzene is prepared, and also a colour-reaction mixture R; this consists of 5 ml. of saturated rhodamine B in benzene plus 10 ml. of 2 per cent uranyl nitrate in benzene. This reaction-mixture is used to give the fluorescence described by Feigl[8] for the higher carboxylic acids, for which, however, it is not altogether specific. To three square 1 ml. cuvettes which fit the Photovolt Corporation's fluorescence meter, one adds 1 ml. of the standard S, 1 ml. of the unknown X, and 1 ml. of benzene (to serve as a blank b). The fluorescence of each is read; they are not the same, because benzene-extracted tissues contain fluorescent substances other than C-18 fatty acids. The fluorescence of the blank is subtracted from that of the unknown, and the result is divided by the fluorescence of the standard with that of the blank subtracted. This gives F_1. To each cuvette is added $0{\cdot}75$ ml. of the reaction mixture, and after stirring with an air jet, the fluorescence of each cuvette is read. The fluorescence of the unknown, with that of the blank subtracted, is divided by the fluorescence of the standard with that of the blank subtracted. This gives F_2; $(F_2 - F_1)$ gives the fluorescence due to the higher carboxylic acids. The primary filter in the fluorescence meter transmits the 365 mμ mercury line, and the secondary filter transmits the 410–480 mμ band. The Photovolt Corporation's photomultiplier, the search unit of which is attached to the fluorescence meter, should operate at its highest multiplication, which is about 10^5.

The fluorescence of the standard is set by the photo-attenuator at some figure between 10 and 30 on the photometric scale of the photomultiplier. Since the concentration of C-18 acids in the standard is $M/100$, $2{\cdot}8\ (F_2 - F_1)$ gives the concentration in mgm./ml. of the higher fatty acids in the unknown. The dry weight of the material in the mince is known, and so the amount of C-18 fatty acid contained in 1 gm. of dried mince can be calculated. The same method can be applied to tissues of any normal or tumour-bearing animal.

Care must be taken that fatty acids from the hands, etc., are not introduced into the standard, the unknown, and the blank. The inhalation of benzene during pipetting is dangerous. The photomultiplier and fluorescence meter are apt to be unstable unless warmed up for 30 min., and the primary shutter of the fluorescence meter must be kept closed until the blank, the standard, or the unknown are in place in the cuvette holder; the primary shutter is then opened for a few seconds, and the fluorescence is read. If the apparatus is used in this way, it is stable and the relation between fluorescence and concentration of C-18 fatty acid is almost linear. When the fluorescence of the unknown is much greater than that of the standard, as in liver and lung, the benzene extracts should be diluted so that the fluorescence meter can be used very nearly as a null instrument.

The standard S used in the fluorescence measurements is a $M/100$ solution of ricinoleic (or oleic) acid in benzene. It contains $2{\cdot}8$ mgm./ml. of fatty acid and 60×10^{17} fatty acid molecules per ml. The expression $(F_2 - F_1) = 280/wd.(X' - b)/(S' - b)$ gives the quantity of higher fatty acids in mgm./gm., where d is the percentage of dry material in the unknown and w is the weight (gm.) of the benzene-extracted tissue. This often gives large values, for example, 5–50 mgm./gm. of dry tissue.

If 1 ml. of the benzene solution of the unknown is dried in a current of air and the fatty acid taken up in m ml. of phosphate buffer at pH 7, its lytic activity can be compared with that of S, $S/2$, $S/4$, etc., treated similarly. If the unknown has the same lytic activity as S/n, the lytic activity of the material in the unknown X is S/mn. The value is usually small, for example, 70 γ/ml. or less, which is partly due to fatty acids not being soluble at pH $7{\cdot}0$. They are probably soluble, however, in the lipoproteins of the surface of the red cell.

Table 1. APPARENT CONTENT OF C-18 FATTY ACIDS IN DRY WEIGHTS OF TISSUE

Normal *C3H* males		Tumour-bearing *C3H* females			
Liver	Lung	Tumour	Liver	Lung	Muscle
22·4 ± 2·2	29·2 ± 3·1	4·5 ± 1·1	37·8 ± 5·2	39·4 ± 3·7	2·2 ± 0·05

The ratio of the value 2·8 ($F_2 - F_1$) found by fluorescence to that found from hæmolytic activity, for example, 5–50 mgm./ml. compared with 70 γ/ml., or at least 70 to 1, might provide the approximate ratio of the total fatty acid in the system to the fatty acid bound at the red cell surface were it not that the surface can become saturated with fatty acid molecules, arranged end-on in the first instance. The mean surface area of the red cell is $1 \cdot 67 \times 10^{10}$ Å.^{2}, and if the number of cells per ml. is 10^8, the total cell surface is $1 \cdot 67 \times 10^{18}$ Å.^{2}. The cross-sectional area of a fatty acid molecule is about 10 Å.^{2}, so $1 \cdot 67 \times 10^{17}$ Å.^{2} of the cell surface can be covered by a continuous film of fatty acid. The standard system contains 60×10^{17} of fatty acid, and so saturation occurs when about 80 γ/ml. is bound. If a system contains more lysin, the bound lysin probably remains at about 80 γ/ml., and the curve obtained by plotting the free fatty acid against that bound, both in γ/ml., shows a discontinuity at the saturation value. If the system contains less lysin than the saturation value, the quantity of lysin bound is proportionately less. This is in agreement with the results of radioactivity measurements with oleic acid[9]. (The fatty acid was wrongly referred to in this paper as its sodium soap, which it is not at *p*H 7; this makes little difference to the conclusion.)

The mean values, with the standard error of the mean, for the C-18 fatty acids in mgm./gm. dry weight of normal liver, normal lung, tumours, livers, lungs and muscle of tumour-bearing mice are shown in Table 1. The values do not have great precision, partly because the standard deviations are large, partly because a difference ($F_2 - F_1$) is a part of the calculation, and partly because the quantity of blood in the tumours and in the tissues, although small because the mice are bled, is not measured. Even heparinized blood when extracted with benzene and treated with the colour reagent gives a small fluorescence (1·0–1·5 mgm./gm. of dry weight) and some fraction of this, depending on the quantity of blood which the tumours or tissues contain, ought to be subtracted. The values for the livers and lungs of the tumour-bearing females are, however, significantly greater than the corresponding values for the males.

The most significant finding is that, at *p*H 7–8·5, homogenates of *C3H* mouse tumours or of the glands of *AKR* lymphomatous mice[2] are usually more lytic than homogenates of normal mouse liver and lung, while benzol extracts of normal mouse liver and lung apparently contain 4–12 times the amount of C-18 (or higher) fatty acids contained in benzene extracts of the tumours, and as estimated by fluorescence. One explanation is that lytic fatty acids are bound to a greater extent to inhibitors in the tissue homogenates than in the tumour homogenates. Another explanation is that normal mouse liver and lung contain small amounts of non-lytic, benzene-soluble, and very fluorescent substances which enter into the reactions for C-18 fatty acids, whereas these substances scarcely occur at all in tumours.

Contribution of Vitamin A and Vitamin A-like Substances

There is some evidence that mouse liver and lung contain two kinds of benzene-soluble substances (see preceding communication). The first are C-18 fatty acids, which fluoresce on the addition of rhodamine *B* and uranyl nitrate when they are excited in the ultra-violet. These acids are hæmolytic, and exist both in normal mouse liver and lung and also in the mammary tumours of the *C3H* mouse. The second kind of substance is also fluorescent, but is non-lytic; it occurs in mouse liver and lung, but not in mouse mammary tumours. It is possibly vitamin A or a related substance. Its concentration and also that of the C-18 fatty acids can be estimated by a combination of fluoroscopy and ultra-violet spectrophotometry, since vitamin A shows an absorption maximum at 330 mμ, while the C-18 fatty acids show absorption between 280 and 285 mμ and not at 330 mμ.

The procedure is as follows: With a Cary No. 11 recording spectrophotometer, the optical density *D* at 330 mμ is found for a series of concentrations of vitamin A in benzene. Beer's law is obeyed between 1 γ/ml. and 50 γ/ml. About 1 gm. of liver is extracted with benzene in a Soxhlet apparatus and is made up to 50 ml. with benzene. By spectrophotometry, and using the calibration curve already obtained for the optical density *D* at 330 mμ of different concentrations of vitamin A, the concentration of the latter in the liver extract is found to be *x* in γ/ml. This step enables the value of *D* to be related to the value of the fluorescence *F*.

The fluorescence *F* of various concentrations of vitamin A in benzene (1 γ/ml., 5γ/5 ml. 10γ/10 ml., etc.) is found with the Photovolt Corporation's fluorescence meter, and a small value for a blank *b* is subtracted from each. The fluorescence of the liver extract is found similarly. *b* being again subtracted, and the extract being diluted suitably with benzene. The fluorescence *F* can then be related to the optical density *D*, the relation being $F/D = 15 \cdot 4 \pm 1 \cdot 0$. Note that the units for both *F* and *D* are γ/ml. wet weight. This method of estimation can be applied to extracts of male and female mouse livers (the females usually being tumour-bearers in these experiments), to extracts of tumours, etc.

The values of 2·8 (F_2–F_1) are then found for extracts of male and of female livers, tumours, etc., as described above; these values are those for the C-18 fatty acid content, and the unit is now mgm./ml. wet weight. In the case of each tissue extract, the value for the vitamin A content (in γ/ml.) is subtracted from the much larger value for the C-18 fatty acid content (in mgm./ml.); the difference can then be expressed in mgm./gm. dry weight, if desired. The concentration of the lytic C-18 fatty acids in mouse liver and lung is always at least 99 times greater than that of vitamin A or vitamin A-like substances, and so it can be concluded that the principal reason for homogenates of *C3H* mouse tumours being more lytic than homogenates of normal mouse liver or lung, although benzol extracts of the latter seem to contain 4–12 times the amount of C-18 fatty acids contained in the benzene extracts of the mouse tumours (estimations by fluorescence), is that the reaction which estimates the lytic C-18 fatty acids also reacts with a very much smaller amount of very fluorescent substances, such as vitamin A.

A number of subsidiary points have been established during these investigations. (1) The vitamin A concentration of *C3H* mouse tumours is very small, that of the livers of male mice is about 1 γ/ml., but that of the livers of tumour-bearing female mice is about ten times greater. (2) The concentration of vitamin A in benzene extracts of mouse livers is not reduced by shaking with 0·1 *M* sodium hydroxide, that is, the substance with an absorption maximum at 330 mμ is either not a vitamin-A acid, or else is strongly bonded to other benzene-soluble substances. (3) Some of the benzene-soluble substances present in mouse liver are taurocholates (absorption between 281 and 293 mμ), tripalmitates (absorption at 281 mμ), and cholesterol, a possible inhibitor (absorption between 280 and 295 mμ); linoleic acid, a typical C-18 hæmolytic fatty acid, has its maximum absorption at 275 mμ in a 1 per cent solution in benzene, and does not absorb at 330 mμ. Present observations throw no light on the extent to which lytic fatty acids are bound to inhibitors in homogenates of normal tissue and homogenates of tumours.

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SHORTENING OF LIFE AND CAUSES OF DEATH IN MICE EXPOSED TO A SINGLE WHOLE-BODY DOSE OF RADIATION

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A SYSTEMATIC investigation has been carried out on the reduction of life-span and causes of death in mice exposed at the age of 30 days to a single whole-body dose of 15-MeV. X-rays. 4,604 mice, half of them male and half female, of an inbred *A* sub-strain were used. The animals were randomly divided into ten dose groups (Table 1); one of these, the control group, underwent the procedure of 'sham' irradiation.

Apart from the single event of the exposure to radiation, all irradiated mice were kept to the end of their lives under the same conditions as the control population. The age at death was recorded for each animal, and an attempt made to establish the cause of death at autopsy.

A full description of the experimental technique and a detailed analysis of the results will be published in the *Proceedings of the Royal Society*[1]; in this article we present only a summary of the results and conclusions.

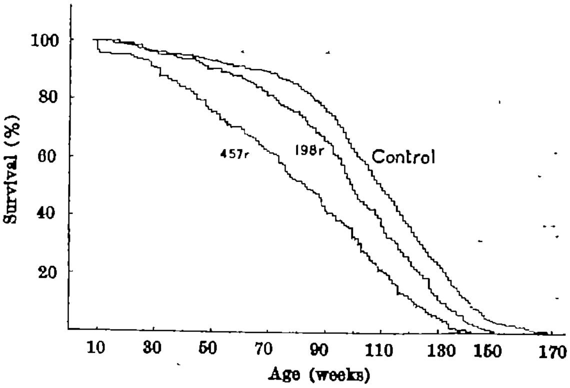

Fig. 1. Survival curves of male mice

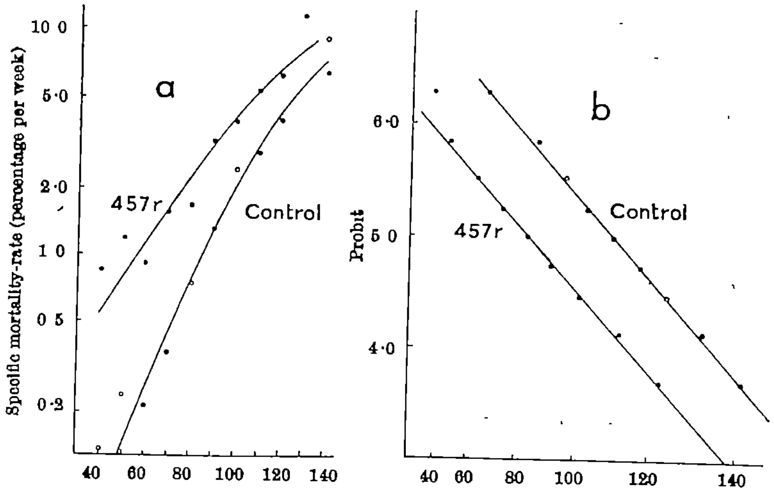

Fig. 2. (*a*) Gompertz plots in 10-week intervals; (*b*) probit as a function of square of age for every decile of the survival curves

Shortening of Life

Survival curves, in which the percentage of the population surviving to a given age is plotted against the age, were drawn for every dose group, counting as 100 per cent the mice alive at the age of 60 days, that is, 30 days after the exposure. Typical survival curves for the male control group as well as for male mice exposed to 198 and 457 r. are given in Fig. 1.

An attempt was made to analyse the survival curves in terms of a Gompertz plot (logarithm of specific mortality-rate versus age). As seen from Fig. 2*a*, the experimental points are rather widely scattered

Table 1. DOSE GROUPS

Dose (r.)	Number of mice	Dose (r.)	Number of mice
0	1,152	457	576
50	576	549	144
98	576	630	140
198	576	703	144
350	576	780	144

and they do not fall on straight lines as might have been expected[2]. On the other hand, a plot of the probit of the percentage survival against the square of age was found to give an excellent linear regression (Fig. 2*b*).

Two effects of radiation are noted from the survival curves: (*a*) a reduction in the median age; (*b*) a small increase in the standard deviation of the distribution of ages at death. We suggest that the latter effect is the result of the greater spread in the ages of onset of different diseases which occurs in the irradiated animals.

We have calculated that this increased spread would give rise to the following variation of the standard deviation with dose:

$$\sigma = [27 \cdot 3 + 0 \cdot 2D + 0 \cdot 001\, D^2$$

where σ is the standard deviation in weeks and D is the dose in roentgens. The observed variation of σ with dose was found to agree closely with this expression.

The main effect of radiation, that is, the shift of the survival curves towards a younger age, was found to be proportional to the dose. If the ages at which the animals from different dose groups reached a given percentage of survival are plotted against the dose, straight lines are obtained. The slopes of these lines are not the same but decrease systematically as one goes from high to low percentages of survival. This is the result of the variation already mentioned of the standard deviation with dose. If a correction for this variation is made, according to the above expression, the slope of all lines becomes the same within the experimental error.

From the displacement of the survival curves relative to that of the control group the amount of life-shortening can be calculated for each dose group. This is plotted against the dose in Fig. 3. It is seen that the points fall closely on a straight line which passes through the origin within the limits of experimental error. The reduction of life-span is thus proportional to the dose, and there is no threshold for this effect.

From the slope of the line the life-shortening is calculated to be 5·66 ± 0·18 weeks per 100 r. Taking into account the relative biological effectiveness of 15-MeV. X-rays (0·81 ± 0·03) this corresponds to 7·00 ± 0·34 weeks per 100 r., if 250-kV. X-rays were used. The relative shortening of life is 38·0 per cent of the median life-span for a dose equal to the *LD*50 (698 r.).

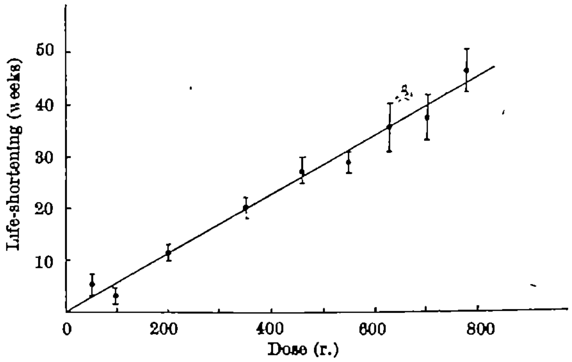

Fig. 3. Shortening of life as a function of dose for mice of both sexes

Separate calculations for male and female mice show a slightly larger life-shortening effect in males than in females, but the difference is within the experimental error.

The life-shortening effect found by us is considerably greater than that observed by other investigators[2]. This may be due to the difference in age at irradiation. We have found previously[4] that at four weeks of age the radiation sensitivity to acute effects is much greater than at other ages. The linear relationship between life-shortening and dose observed

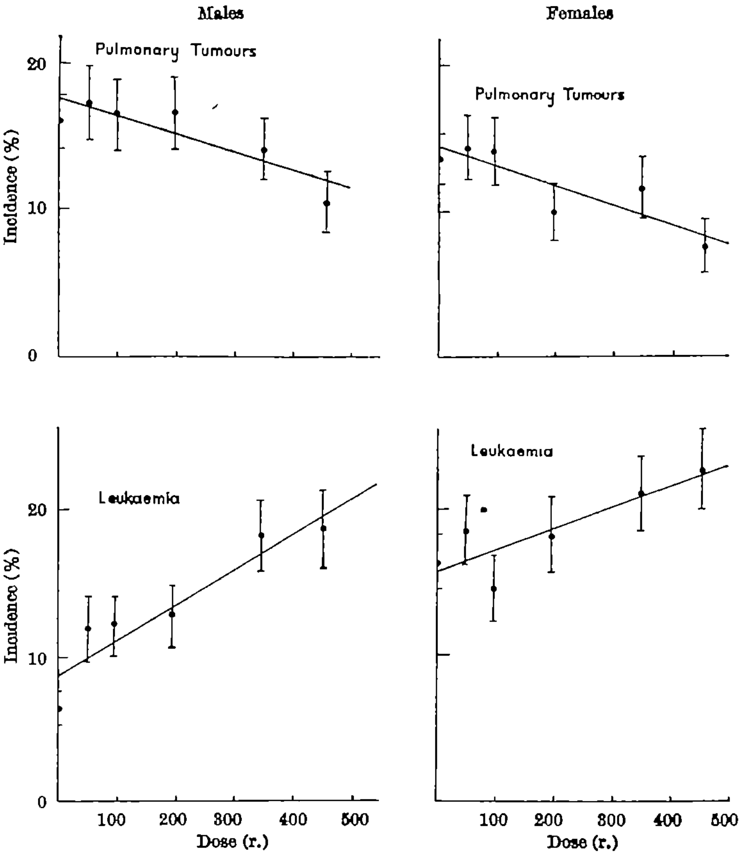

Fig. 4. Percentage incidence of deaths from leukæmia and pulmonary tumours, as a function of dose

Table 2. GROUPING OF CAUSES OF DEATH

Cause	Diseases included
Leukæmia	thymoma, lymphatic and myeloid leukæmia, lymphadenoma, lymphosarcoma
Pulmonary tumours	a few benign adenomata, but mostly adenocarcinomata
Liver tumours	hepatoma, hæmangiomata and secondary deposits from unknown primary tumour
Ovarian tumours	some benign cystadenomata, but mostly granulosa cell tumours
All other tumours	carcinomata or sarcomata not included in the above groups
Pulmonary	degenerative, infective or hæmorrhagic conditions of lung ;
	generalized infection, for example, staphyloccal septicæmia
Renal	mechanical, infective or degenerative conditions in urinary tract
Liver	degenerative, infective or infiltrative conditions of liver
	miscellaneous causes, for example, aneurysm
Intestinal	hæmorrhage, obstruction or infection
Undiagnosed	undiagnosed cause of death, due to lack of visible pathological changes, mouse eaten or autolysed ; this group may include many different diseases

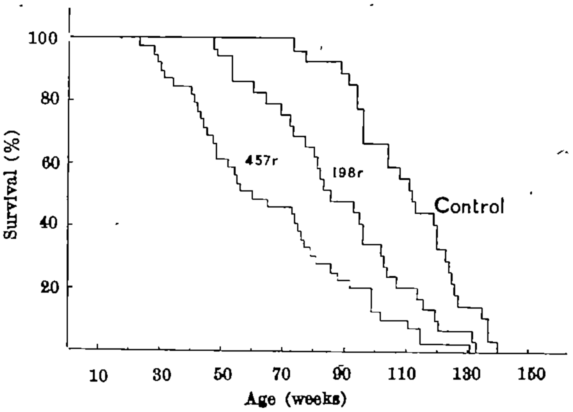

Fig. 5. Age distribution of deaths from leukæmia in male mice

by us, and the lack of a sex difference in this effect, may also be characteristic of the young age at irradiation. Experiments are now in progress to determine the dependence of life-shortening on age at irradiation.

The fact that the corrected survival curves are parallel to each other after the 'plateau' region, suggests that life-shortening is the result of the loss of a few weeks of early life and not of a contraction of the time-scale of the animal's life. From the point of view of survival the irradiated mice behave exactly like their non-irradiated mates of an older age, and only in this sense can one claim that radiation causes premature ageing by an amount proportional to the dose.

Causes of Death

Apart from obtaining a quantitative relationship between shortening of life and dose, the aim of the investigation was to establish whether the reduction of life-span is the result of induction by radiation of certain specific diseases or of an advancement of all natural causes of death. For this purpose a full post-mortem examination has been carried out on each animal which died to try to establish a cause of death, according to certain criteria fixed at the start of the investigation. Random samples of tissues were also taken for histological study. In the final analysis all causes of death were divided into 10 groups (Table 2), which include five tumour and four non-tumour groups. The tenth group, which comprises about 20 per cent of all deaths, contained the cases in which no definite diagnosis could be established.

In view of the much smaller numbers of mice in the highest dose groups, a detailed statistical analysis was carried out only for the six lowest dose groups, 0–457 r.

The percentage incidence of a given cause of death was found, in most cases, to vary very little with dose, but some diseases showed a definite increase and others a definite decrease with dose. The most pronounced absolute changes are shown in Fig. 4 ; these are an increase with dose in the incidence of leukæmia and a decrease in pulmonary tumours. All four plots give reasonably good linear relationships between incidence and dose.

Apart from the change in incidence, the distribution in time of deaths from different causes turned out to be of great interest. 'Survival curves' were drawn for each cause of death in a similar way as in Fig. 1 for deaths from all causes. An example of such a curve, for leukæmia in males, is shown in Fig. 5. From these curves the median age for the occurrence of each disease was determined. A plot of such median ages versus dose is shown in Fig. 6 for the same two diseases as in Fig. 4. Fig. 6 also contains the lines for the median ages at death from all causes. It is seen that while all causes of death were advanced by radiation, some were advanced faster than others ; in particular, leukæmia was advanced more and pulmonary tumours less than the average.

A detailed analysis has shown that there exists a definite correlation between the acceleration of a given

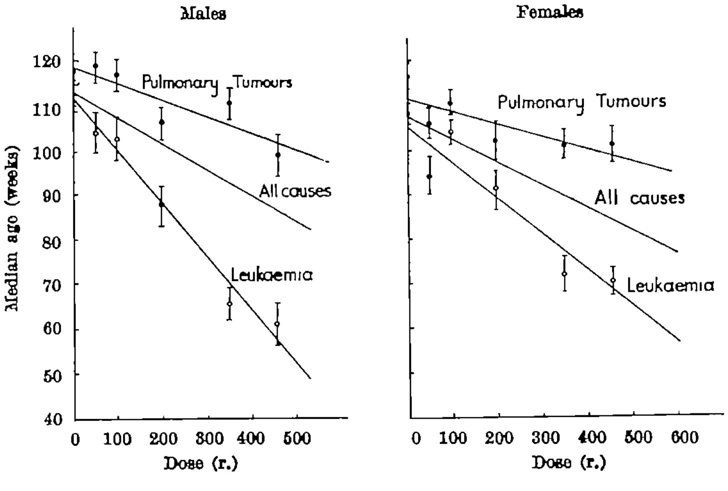

Fig. 6. Median age at death from different causes as a function of dose. The experimental points were omitted for the lines relating to all causes of death

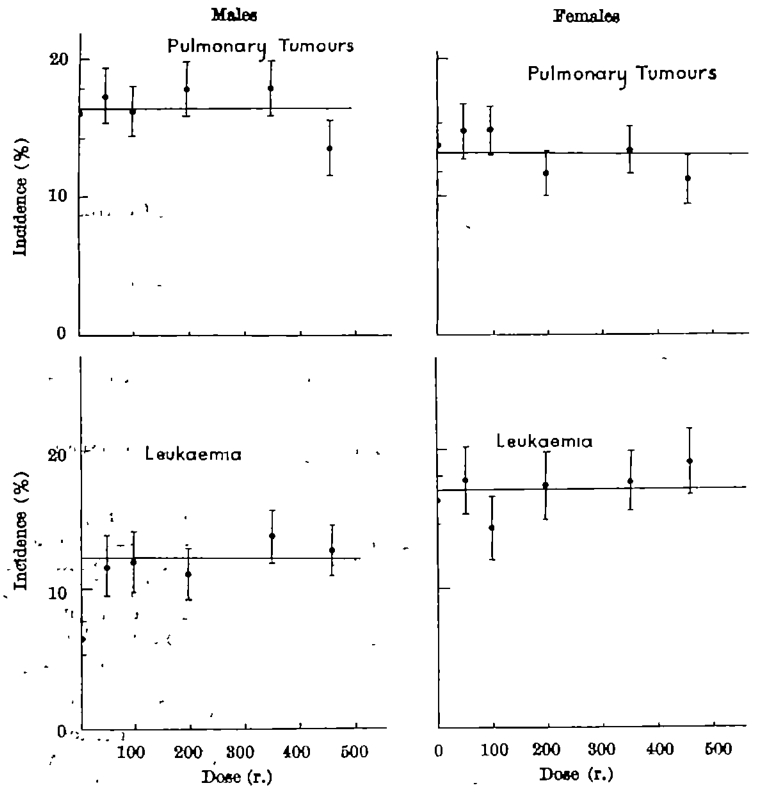

Fig. 7. Percentage incidence of deaths from leukæmia and pulmonary tumours, corrected for the different rates of advancement of these diseases

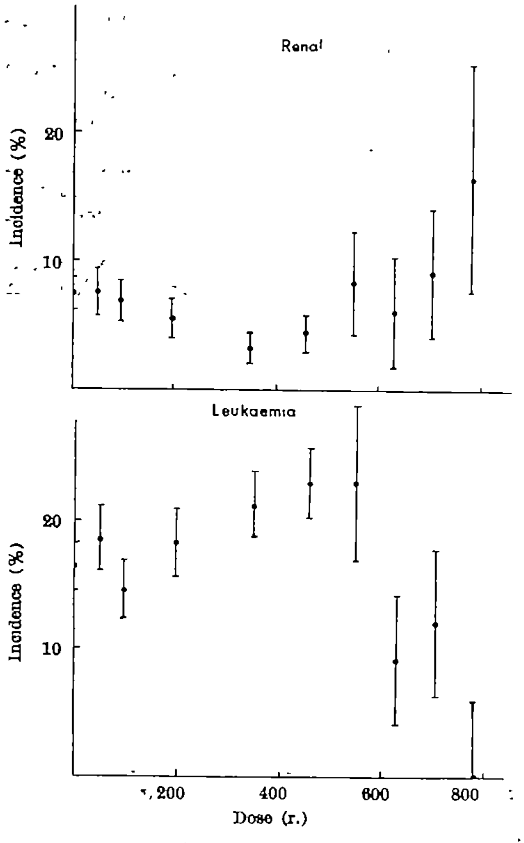

Fig. 8. Percentage incidence of deaths from leukæmia and renal diseases in female mice, showing effect of high doses

cause of death and the rate of change of the percentage incidence with dose. This correlation suggests that the change in incidence is merely the result of the changed times of onset of diseases, since an animal which would have normally died of one disease will die earlier of another disease which is more accelerated by radiation. An actuarial correction was made to take into account the change in time of onset of the different diseases, and the corrected incidences have indeed been found to be independent of dose. This can be seen from Fig. 7, which should be compared with Fig. 4. We conclude, therefore, that the intrinsic probability of any one disease occurring is the same in irradiated as in control animals, the main effect of radiation being an advancement of all causes of death but at different rates. Which disease is more advanced may depend not only on strain characteristics, but also on the age of the animal at irradiation. The pathogenesis of diseases occurring later in life and contributing to death may be laid down at different ages in life. In this investigation, in which the irradiation was given at the age of 30 days, when the hæmopoietic tissue is most active, leukæmia was most advanced by radiation. Exposure at another age may well bring to the fore a completely different disease.

In the high-dose groups another effect was observed, namely, a decrease in incidence of all neoplastic diseases, with a corresponding increase in the incidence of non-neoplastic diseases. This can be seen from Fig. 8, which shows the variation with dose of the incidence of leukæmia and renal diseases. A decrease in incidence of neoplastic diseases at high doses has been observed by others[5]; a similar effect was found in the study of genetic mutation-rates[6]. It may be due to the lethal effect of radiation on tissue; a large dose may kill the cells, or may prevent the initial change in the cells, which would have later become manifest as a neoplastic condition.

The main conclusion from this work is that the shortening of life produced by radiation is due not to the induction of specific diseases but to the bringing forward in time of all causes of death, without any intrinsic change in their relative probabilities. From this point of view radiation may be considered as an ageing factor. It cannot, however, be identical with natural ageing, since the relative ages of onset of diseases are not the same in the irradiated as in the control populations.

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NEW FINDS AT OLDUVAI GORGE

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IN *Nature* of December 17, 1960, p. 1050, I reported the discovery of the bones of a hominid foot as well as some other specimens, in deposits of Bed I at Olduvai, but in a geological stratum lower than that which yielded the skull of *Zinjanthropus* in 1959.

The deposit at site *F.L.K.N.N.* I, which yielded the foot bones, the few hand bones, some tiny skull fragments, the two clavicles and the 'lissoir', has since then yielded the greater part of a hominid mandible (see Fig. 1) (found by my son, Jonathan Leakey, on November 2), as well as parts of two hominid parietals (see Fig. 2).

From the mandible, it is possible to estimate that the age at death of this individual was about twelve years. This is on the basis of present-day tooth eruption, since the second molars are in occlusal position but little worn, while there is no sign whatsoever of the eruption of the third molars. It is realized, of course, that in Lower Pleistocene times growth may have been more rapid, and that this jaw may perhaps represent an individual of less than twelve years.

The new mandible can be regarded, almost certainly, as belonging with the two parietals, the foot bones, some of the hand bones, and one of the clavicles. Since these remains represent a hominid which is stratigraphically earlier than *Zinjanthropus*, they are of considerable scientific interest.

Although detailed study has not yet been undertaken, the following facts may be placed on record:

(*a*) The overall dental pattern does not appear to be compatible with the type seen in *Zinjanthropus* or any other Australopithecine, and it seems possible that we are dealing with a quite distinct type of early hominid.

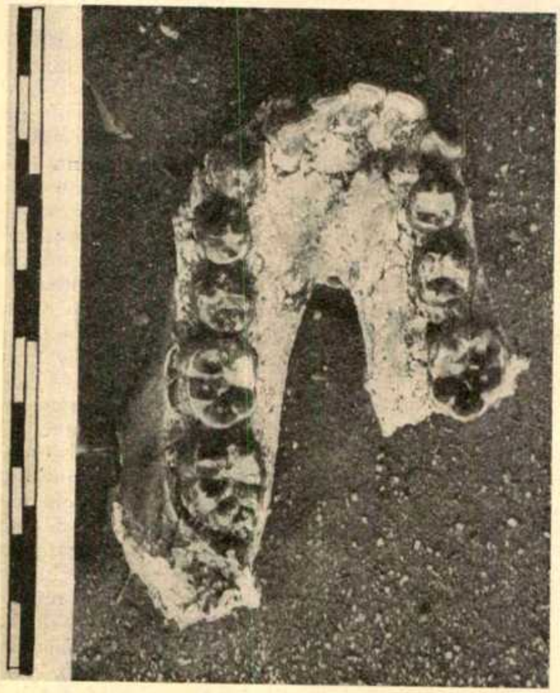

Fig. 1. The new jaw from site *F.L.K.N.N.* I Olduvai

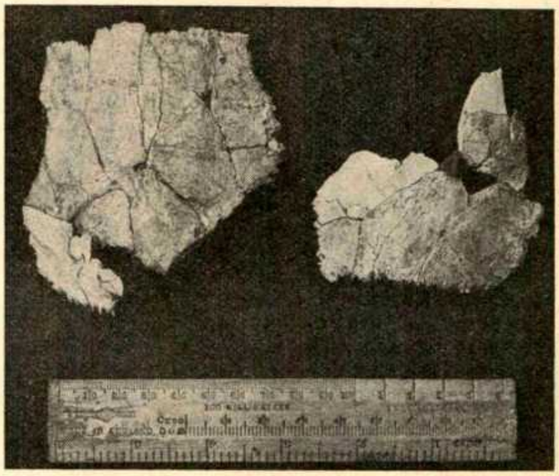

Fig. 2. Two frontals of a juvenile. They are a little larger than those of *Zinjanthropus*, and probably belong with the mandible

(*b*) The lower canines, while relatively large, are wholly hominid in type and morphologically quite unlike the canines of pongids, but recall *Australopithecus*.

(*c*) The lower incisors are also hominid in their general morphology although they do, to some extent, recall the lower incisors of *Proconsul*.

(*d*) The premolars are remarkable and are unlike what is normally to be seen in the Australopithecinæ. The anterior–posterior length in both the third and fourth premolars is greater than the bucco–lingual width, while the fourth premolar has very well-marked cuspules bordering the edge of a large posterior fovea.

(*e*) The first molar is well worn and has a general cusp pattern reminiscent of what can be seen in some recent Australian Aborigines, but it is of course larger.

(*f*) The second molar is much longer than the first and is remarkably elongate.

The mandible was unfortunately broken prior to fossilization, and part of the right ramus has been distorted and made to appear much nearer to the left half than is really the case. The *corpus mandibulare* is very massive indeed. Unfortunately, the lower margin of the mandible is missing, so that it is not possible to say anything about the lower rim of the mandible or of the symphyseal region.

The two parietals are especially remarkable because, although they apparently belong to a young individual only twelve years old (or less), they are larger than those of *Zinjanthropus*. They are remarkably thin, and exhibit no sign of a sagittal crest or of any marked temporal line. The lack of both these may, perhaps, be due in part to the youthful age. Nevertheless, these parietals suggest that we are dealing with a hominid with a larger brain capacity, as well as somewhat less specialized, than *Zinjanthropus*.

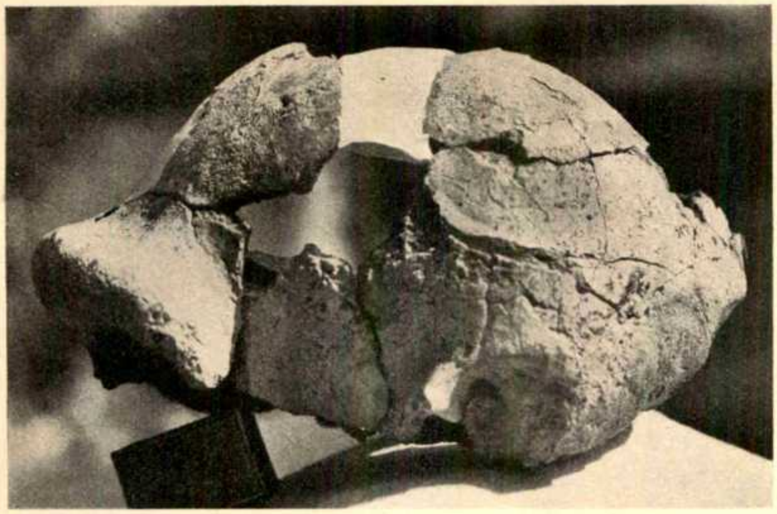

Fig. 3. Profile view of the skull of a man from Bed II Olduvai (*L.L.K.* II) found at a level which yielded abundant stone tools of Stage 3 of a Chellean culture. The skull is 209 mm. long

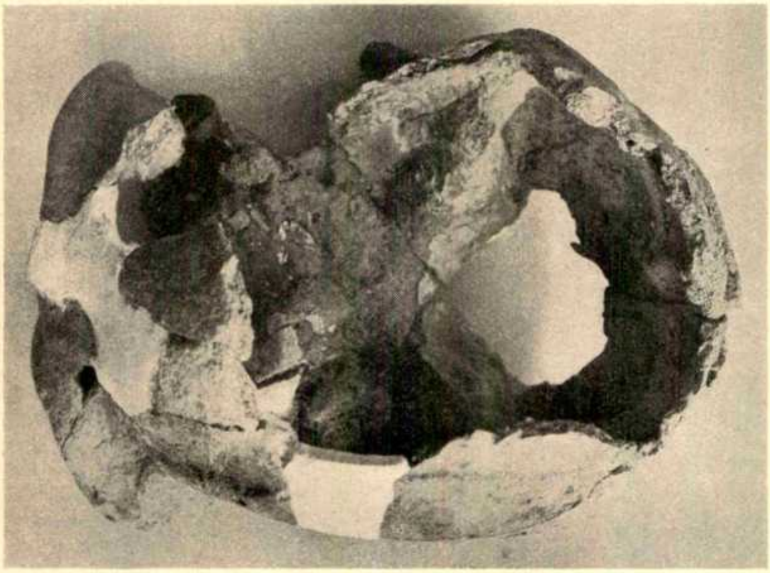

Fig. 4. View of the skull of Fig. 2 from above, to show the brow ridge area and other characters

At the time of writing the communication published in *Nature* of December 17, very few stone tools had been found in this lower level; since then, more have been found.

Another discovery of outstanding interest was made on December 2. This consists of the brain case (see Figs. 3 and 4) of a hominid from Bed II at Olduvai, at Site *L.L.K.* This skull was found at a stratigraphic horizon which yields quantities of stone tools of stage 3 of the Chellean culture. It may reasonably be assumed that we have, at last, found a skull representing the makers of the Chellean culture.

The Chellean culture was the first recognized stone-age culture ever to be officially accepted by science more than a century ago, but in all the years that have followed, no authentic find representing 'Chellean man' has been made other than two teeth found at Olduvai in 1954, and reported in *Nature* in 1958. In this connexion it must be noted here that *Atlanthropus*, found by Prof. Arambourg in North Africa, was, in the first instance, placed on record as being associated with Chellean tools; but this has since proved to be incorrect, since the cultural material found with them is quite clearly of Acheulean type.

This new skull from Olduvai, which comes from the well-established Chellean horizon in the gorge, is remarkable in a number of respects. It has a number of resemblances (although some of them are only superficial) to the Pithecanthropines. In other characters, however, the new skull shows considerable resemblances to the Steinheim skull from Germany, a specimen which is usually regarded as contemporary with the early phases of the Acheulean hand-axe culture and assigned to the genus *Homo*. The new skull also shows certain resemblances to two well-known African fossil skulls, those from Broken Hill and Saldhana, respectively. It is very large—209 mm. long and 150 mm. wide—and while the vault of the skull is low compared with present-day man, it is high compared with the fossil skulls from Java and Pekin. No discussion of points of detail will be attempted at present.

The third discovery made in recent months is that of an exceptionally rich living floor of a late stage of the Oldowan culture, some 20 ft. higher in Bed I than the *Zinjanthropus*-level and immediately underlying the 'marker bed' at the top of Bed I. The cultural material in this new floor is associated with a very rich fauna and is of special interest because it provides an intermediate stage between the ordinary Oldowan and stage 1 of the Chellean, as found at sites such as *B.K.* II. Moreover, the study of the fauna found here indicates that, at this level, man had more skill in hunting the large adult animals living at that time than is shown at the *Zinjanthropus*-level.

I wish to thank the National Geographic Society, which has enabled us to work in the field continuously since February 1960, with a large party. I also owe special thanks to my wife and to my son Jonathan, and to my senior assistant, Heselon, on whom the bulk of the work in this very long season has fallen. I am grateful to the Geological Survey of Tanganyika for making available the services of Dr. Pickering to carry out geological mapping on the sites, and on the Gorge as a whole. I also thank the many individuals, too numerous to name, as well as the Wenner Gren Foundation and the Wilkie Foundation, who have helped in a variety of ways to make this further season possible.

LETTERS TO THE EDITORS

ASTROPHYSICS

Oscillation Broadening of Lines from Ionized Elements in Stellar Spectra

THE well-known sources of line-broadening in stellar spectra may be summarized in two main groups. (1) Broadening resulting from the finite length of the wave-trains emitted by the atoms. The length is determined by (*a*) the finite life-time of the excited level, that is, radiation damping, and by (*b*) abrupt phase-changes in the emitted wave caused by perturbations of the emitting atoms by other particles, that is, collision damping and Stark effect. (2) Broadening resulting from Doppler effect of different types. The Doppler velocities may be of a random character, for example, thermal or turbulent motions of emitting atoms, resulting in a line profile of gaussian shape, or the broadening may result from the integration of contributions to the line from different parts of a rotating star, an expanding gas shell or a pulsating star. In addition, broadening or splitting of lines by magnetic fields (Zeeman effect) and by coupling between the nuclear spin and the angular momentum of the electrons of the emitting atom (hyperfine structure) can occur.

The aim of this communication is to direct attention to a broadening mechanism which has not been mentioned in the literature.

Abrupt disturbances introduced in a plasma will give rise to electron oscillations at, or near, the Langmuir frequency. To a first approximation, the electrons will perform harmonic, linear oscillations about a position of equilibrium, with a linear velocity $V_e = V_{oe} \cos \omega_p t$, where $\omega_p \sim$ Langmuir frequency, the restoring force being the field set up by the electrons themselves.

It will be shown in a subsequent paper (in preparation) that ions in the field set up by the electrons will oscillate with the same frequency as the electrons and with a maximum velocity:

$$V_{oi} = \frac{Z.2}{A.1{,}840} \cdot V_{oe}$$

where Z is charge of ion in units of electronic charge and A is mass of ion relative to the mass of the hydrogen atom.

This means, if the state of excitation of the oscillating ions is such that they give rise to an absorption or emission line, the observed line will, in addition to the width determined by the well-known sources mentioned above, show a broadening caused by Doppler velocities of the form $V = V_{oi} \sin \omega_p t$.

This broadening effect is proportional (1) to the oscillatory velocity of the electrons (or, to the magnitude of the oscillations) and (2) to the charge to mass ratio of the ions in question.

The line profile may be determined qualitatively by treating the phenomenon as a frequency modulated signal at a rest-frequency $\omega_o = 2\pi c/\lambda_o$ with a modulation frequency ω_p (the Langmuir frequency) and a modulation index $\beta = \dfrac{V_{oi}.\omega_o}{c.\omega_p}$, resulting in a line pattern consisting of frequency components centred at $\omega_o \pm n.\omega_p$, where $n = 0, 1, 2, 3. . . .$

The amplitude of the nth component is given by the Bessel function of the first kind and nth order with β as argument. In addition, each component of the line pattern is subject to conventional broadening.

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Deflexion of Light in the Gravitational Field of the Sun

ACCORDING to the whirl theory of light and matter[1], the whole physical world is the manifestation of Maxwell's electromagnetic waves. The fundamental particles of matter, and the photons, are represented by integral solutions of Maxwell's equations *in vacuo*, in cylindrical co-ordinates. For the former the integral is taken from a very low limit of the variable parameter of the integrant solution to infinity, while in the latter the interval of integration is much smaller. The forces are determined from the standard equations of classical dynamics as the derivatives of the exchange integrals of electromagnetic fields of interacting entities with respect to parameters defining their mutual position. The necessary condition of physical validity of these forces is the synchronism between the fundamental frequencies of the entities or their sub-harmonics. In the case of gravitation the sub-harmonics are due to the 'micro-precessions' of the entities, of about $8 \cdot 3 \times 10^{-7}$ sec.$^{-1}$, caused by their inherent eccentricity of about 10^{-10} h/mc. In this very slow motion the wave-length of de Broglie's waves (which are inherent in these solutions of Maxwell's equations) is about 10^{12} cm. They are called the 'macrowaves'. The macrowaves produce the mutual synchronization of micro-precessions. The resultant macrowaves of thus synchronized particles constituting large axially symmetrical bodies, such as the Sun or the planets, form co-axially revolving wave systems with radial standing waves. The orbits of planets are situated in the troughs of the latter.

The whirl theory has permitted, so far, the calculation of fourteen physical constants from five observation data (c, h, e, the mass of the proton m_+, and the gravitational constant γ_g). These included the mass ratio of the proton and electron, the masses of the neutron and of the light nuclei ${}_1H^2$, ${}_1H^3$, ${}_2He^3$, and ${}_2He^4$, the distance from the Sun (ref. 1*k*), and the period of rotation and of sidereal motion, of Mercury, the relationship between the periods of rotation of planets and their distances from the Sun, and the magnetic moment of the Earth. The results of the calculations agreed very well with the observation data. The period of rotation of Venus (20 hr. 48 min.) which followed from these results appeared too small at that time (1950), but it was later confirmed by the radio-astronomical observations in the United States (22 hr. 16 min) disclosed in 1956 [2]. The whirl theory also permitted an explanation on the basis of Maxwell's electrodynamics of the growth of crystals from a grain of crystalline substance, the chemical catalysis, and the shifting to the red of spectral[1j] lines of remote nebulae.

Recently, I applied this theory to the calculation of the angle of deflexion φ of a light ray by the field of gravitation of the Sun, using the formulæ :

$$\varphi = \frac{\gamma_{pg} M}{c^2 D}\left[\left(\frac{D}{R}\right)^2 + 1\right]^{1/2} \cong \frac{\gamma_{pg} M}{c^2 R}$$

and
$$\gamma_{pg} = \left(\frac{q_p}{q_+}\right)^{1/2}\left(\frac{q_+}{q_-}\right)^{3/2}\gamma_g$$

where $q_p \equiv 2\pi/\lambda_p \quad q_+ \equiv 2\pi/\lambda_+ = m_+c/\hbar$

$$q_- \equiv 2\pi/\lambda_- = m_-c/\hbar$$

is the wave parameter of the photons in the deflected ray, of the proton, and of the electron, respectively ; m_+ and m_- are the mass of the proton and the electron, respectively ; c is the velocity of light, $\hbar = h/2\pi$ is the Planck constant ; M is the mass of the Sun, D is the distance of the Earth from the Sun, R is the nearest distance of the deflected ray from the centre of the Sun, γ_g is the gravitational constant between material masses, γ_{pg} is the gravitational constant between the material masses and the photon (the mass of the latter equals $\hbar q_p/c$).

The first formula has been derived from classical mechanics. The second formula can easily be deduced from formulæ (13·1) and (13·2) (ref. 1*j*). On substituting the numerical values, I obtain for the angle of deflexion $\varphi = 6{\cdot}2 \times 10^{-6}$ and $8{\cdot}75 \times 10^{-6}$ for the wave-length in the deflected ray 0·8μ (red light) and 0·4μ (violet light), respectively. The average (root mean square) between these two values is about $7{\cdot}8 \times 10^{-6}$.

Thus, according to the whirl theory, the angle of deflexion must depend upon the colour of the observed ray. This can only be verified by observation when the next opportunity (the solar eclipse) comes. It will be noticed that the average angle of deflexion calculated here is very near that obtained on the basis of Einstein's general theory of relativity. This may be due to the smallness of curvatures of geodesic lines assumed by Einstein in his calculations, thus bringing on this occasion the co-ordinate system of the general theory of relativity very near the co-ordinate system of the special theory of relativity which is implied in the whirl theory owing to the relativistic invariance of Maxwell's equations.

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[1] *a*, Japolsky, N. S., *Phil. Mag.*, 19, 984 (1935); *b*, 20, 417 (1935); *c*, 20, 641 (1935); *d*, 22, 537 (1936); *e*, 22, 1003 (1936); *f*, *Proc. Nat. Acad. Sci. India*, 9 (4), 213 (1939); 10 (1), 19 (1940); *g*, *Nature*, 137, 582 (1936); *h*, 154, 20 (1944); *i*, 159, 580 (1947); *j*, *Pakistan J. Sci. Res.*, 111 (Oct. 1949); *k*, 1 (Jan. 1950); *l*, *Bull. Soc. Belge de Phys.* (Jan. 1958).

[2] Kraus, John D., *Nature*, 178, 687 (1956).

PHYSICS

Recombination through Electron–Electron Collisions

IN dense plasmas recombination through electron–electron collisions can be the major cause of loss of free electrons. The mechanism is akin to three-body ionic recombination but differs because of the long-range Coulomb forces involved, because the bound states cannot be treated as forming a continuum and because of the effect of radiative transitions.

Suppose that the ions are protons and suppose also that the temperature is such that the mean thermal energy is much less than the first excitation energy of hydrogen. Radiative recombination is slow but highly excited hydrogen atoms are rapidly formed through electron–electron collisions :

$$H^+ + e + e \rightarrow H' + e$$

These atoms may be ionized by the reverse process ; or they may gain or lose internal energy by suffering superelastic or inelastic collisions with electrons, or by absorbing or emitting photons. The passage of an electron from a state of the continuum to the ground state of an atom is clearly in the nature of a random walk during the course of which many different states may be entered.

Now the rates at which atoms in a particular excited state are produced or destroyed per unit volume by the collision and radiative processes mentioned are very much greater than the rate at which the minute number-density of atoms in that state changes. For given number-densities of free electrons and of normal atoms and given temperature it is hence possible to calculate the number densities of atoms in the various excited states by setting up and solving the matrix equation expressing the close equality of the production and destruction rates. It is then a simple task to calculate the rate at which the number-density of normal atoms is increasing. Since the number-density of excited atoms is, relatively, very small, this rate may be taken to be the rate of disappearance of free electrons. The recombination coefficient, $\alpha(n)$, defined by the equation :

$$\frac{dn}{dt} = -\alpha(n)n^2$$

in which n is the number-density of free electrons, may hence be found.

Calculations have been carried out to cover various conditions. Details will be published elsewhere. Some representative results are presented in Fig. 1. This refers to a plasma at a temperature of 16,000° K. which is almost completely ionized and which is optically thin so that the absorption of the emitted photons may be neglected. If n is less than about 10^{11}/cm.^{3}, $\alpha(n)$ is only about 3×10^{-13} cm.^{3}/sec., which is the value corresponding to radiative recombination. However, if n is greater than 10^{14}/cm.^{3}, recombination through electron–electron collisions is dominant and $\alpha(n)$ is very much larger ; indeed, as n tends to infinity $\alpha(n)$ tends to $9 \times 10^{-28}n$ cm.^{3}/sec. The peculiar slight change in the slope of the graph when n is between 10^{15}/cm.^{3} and 10^{16}/cm.^{3} arises because the ratio of the actual to the Saha-equilibrium population in the second quantum-level becomes comparable with its limiting value, unity, here ; and consequently, the rate at which this ratio increases as n increases ceases being rapid and becomes slow. It may be remarked that while collisions with electrons control the rate of degradation of excited atoms in the high-density limit, bound–bound radiative transitions are generally extremely important.

Several experimental studies[1] have been made on the decay of pulsed condenser and other discharges in which n was about 10^{16} to 10^{17}/cm.^{3}. Values of $\alpha(n)$ of about 10^{-13} cm.^{3}/sec.–10^{-11} cm.^{3}/sec. have been

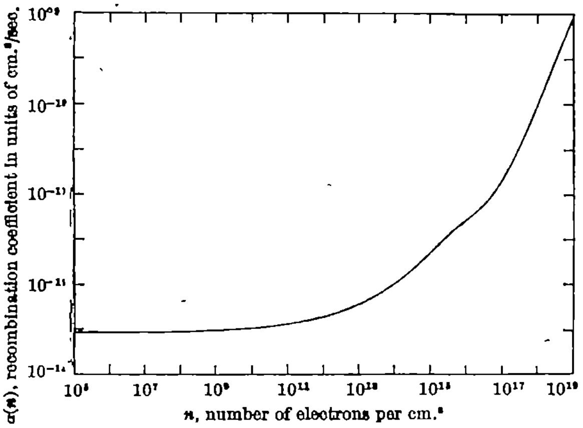

Fig. 1

deduced. Some of these values are far less than would be expected from the theory outlined here. However, the experimental studies concerned give no information on the recombination coefficient. This is so great that the number-density of free electrons simply followed the changing equilibrium as the discharge cooled.

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[1] Loeb, L. B., *Basic Processes of Gaseous Electronics*, 559 (Univ. of California Press, 1955).

A New Type of Phenomenon of Flow Birefringence in Macromolecular Solutions

During an investigation of solution states of methyl cellulose in aqueous systems, an unexpected new phenomenon was noted. The observed optical anisotropy as a function of the velocity gradient in the systems changed anomalously, as shown in Fig. 1 (solid lines). Reproducibility of the variations both of the degree of birefringence and the angle of extinction versus the rates of shear were within experimental error.

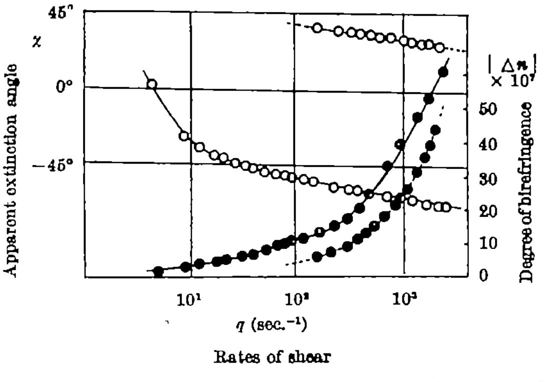

Fig. 1. Variations of the optical anisotropy and the degree of birefringence vs. the rates of shear: original methyl cellulose/water solution (dotted lines); aged methyl cellulose/water solution (solid lines).—○—, optical anisotropy (χ); —●—, birefringence (Δn)

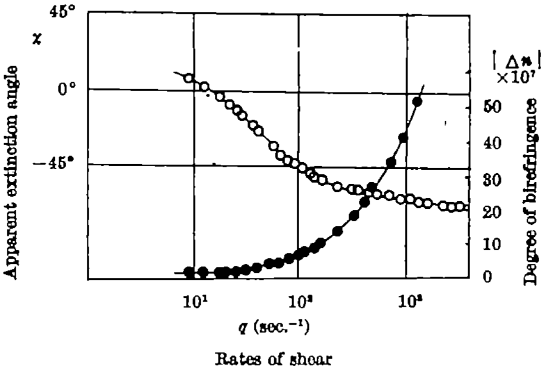

Fig. 2. Variations of the optical anisotropy and the degree of birefringence vs. the rates of shear in a methyl cellulose/methyl alcohol/water system

Dilute aqueous solutions of 'Methocel' (Dow Chemical's powdered methyl cellulose: U.S.P. grade, *MC*-4,000 c.p., average molecular weight determined by viscometry about 140,000) were used in this investigation.

The dotted lines in Fig. 1 represent the normal flow birefringence obtained in the fresh solution, for comparison with the new phenomenon observed after the solution has been aged for two or more months at room temperature (about 30° C.).

A similar marked anomaly was noted in aged aqueous solutions even in fresh aqueous solution of methyl cellulose containing 50 volume per cent of methyl alcohol (see Fig. 2). With a mixed system of ethyl alcohol and water, an effect similar to that with methyl alcohol/water was noted.

It is difficult to find a theoretical explanation of the phenomenon. It seems to be connected with the fact that the solution states of aqueous methyl cellulose are not stable, in spite of the non-ionic nature of the polymer structure. The association of the chain molecules proceeds mainly due to dehydration of the molecules after the solution has been aged[1,2].

Further additional information has been obtained about this effect, using a solution which was mixed with considerable quantities of methyl alcohol in order to regulate the solvent power or the degree of dehydration of the polymer chains. The polymer concentration of the solutions was 1 per cent throughout the investigation, and no essential difference was found between the results obtained in the aged solutions and in the mixed systems. Both effects can probably be attributed to inter- and intramolecular hydrogen bonds between exposed hydroxyl groups of the polymer chains suffering dehydration during ageing, or artificially by the addition of a strong hydrophilic substance such as the alcohol.

The anomalous variations of the extinction angle with increasing rates of shear are still unexplained, and work is in progress to shed more light on the problem.

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[1] Heymann, E., *Trans. Farad. Soc.*, **31**, 846 (1935).

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The Relativistic Theory of the Fresnel Drag Coefficient

In this communication I give a somewhat more general discussion of the Fresnel drag coefficient than is usual, with the view of clarifying the relation between two expressions for it, which occur in the literature of special relativity. These expressions are normally derived separately and their relationship has recently been discussed by Synge[1]. I take a further step here, by showing how both results can be obtained from a single argument. This procedure directs appropriate attention to step (6) (see below), which is the crucial point in both cases. The necessary background may be outlined as follows:

Suppose a medium has velocity v parallel to the x-axis of a reference system S. If light passes through the medium parallel to this direction, let its phase velocity be u_m relative to S. Assume that $u_m = c/n(\nu_m)$, where $n(\nu_m)$ is the refractive index of light of frequency ν_m in the medium, provided the medium is at rest in S (that is, $v = 0$). Then in the general case ($v \neq 0$):

$$u_m = c/n(\nu_m) + kv \qquad (1)$$

Here k is a coefficient which was once supposed to measure the extent to which the æther is dragged along by the medium (Fresnel, 1818). The special relativity theory also leads to (1). For k, it yields the result:

$$k = 1 - \frac{1}{n(\nu_0)^2} + \frac{c}{v}\,\frac{\nu_0}{n(\nu_0)^r}\left\{\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}\right\}_{\nu = \nu_0} \qquad (2)$$

Here $r = 1$ in the case of water flowing at velocity v in a tube fixed in S, and $r = 2$ for a block of glass moving with velocity v. These are the two cases usually described.

Consider now a uniform transparent medium in uniform motion with velocity v parallel to the x-axis of an inertial system S. The medium is contained in a transparent vessel the walls of which can unfold during the motion of the medium, so that the rear face of the container (which is perpendicular to the x-axis) moves with a velocity $w \leqslant v$. The optical properties of the medium in the container are, however, kept homogeneous and constant in time by feeding additional material through a tube leading to the rear of the container. This means that we assume the following idealized situation: At any time t, contact between the rear surface of the container and the medium is about to break due to the difference in velocity. But the gap $(v - w)\mathrm{d}t$, which one would expect to develop in time $\mathrm{d}t$, is filled in by additional material having already v as its x-component of velocity. Contact between the rear surface and the medium is assumed to be maintained in this way; different layers of material are involved in the contact at different instants. This situation cannot be realized exactly, but my main interest is in the two limiting cases, which can be realized.

Let the reference system in which the medium is at rest be S'; that in which the rear face is at rest, be S''; the velocity of S'' as observed in S' be w'; the frequency of the light when moving in vacuum and observed in S'' be ν_0'' and the frequency when moving in the medium and observed in S'' be ν_m''. I shall adopt this type of notation also for the frames S and S' and for the phase velocity of the light, which is denoted by u_0 for vacuum and by u_m for the medium.

By the relativistic velocity addition:

$$u_m = \frac{u_m' + v}{1 + u_m' v/c^2} = \frac{c/n(\nu_m') + v}{1 + v/cn(\nu_m')} \qquad (3)$$

It has been assumed that $u_m' = c/n(\nu_m')$, where $n(\nu_m')$ is the refractive index of the medium for waves of frequency ν_m'. Suppose now that if g is a function of order v/c, we have, to first order in v/c:

$$\nu_m' = \nu_0(1-g) \qquad (4)$$

It then follows that:

$$\frac{1}{n(\nu_m')} = \frac{1}{n(\nu_0-\nu_0 g)} = \frac{1}{n(\nu_0)} - g\nu_0\left\{\frac{\mathrm{d}}{\mathrm{d}\nu}\left[\frac{1}{n(\nu)}\right]\right\}_{\nu = \nu_0}$$

$$= \frac{1}{n(\nu_0)} + \frac{g\nu_0}{n(\nu_0)^2}\left\{\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}\right\}_{\nu = \nu_0}$$

Substituting in (3), one finds, to first order:

$$u_m = \frac{c}{n(\nu_0)} + kv$$

$$\text{where } k \equiv 1 - \frac{1}{n(\nu_0)^2} + \frac{c}{v}\,\frac{\nu_0}{n(\nu_0)^2}\,g\left\{\frac{\mathrm{d}n(\nu)}{\mathrm{d}\nu}\right\}_{\nu = \nu_0} \qquad (5)$$

Here k has the form of a Fresnel drag coefficient. It remains to calculate g.

From the expressions for the radial Doppler effect:

$$\frac{\nu_m''}{\nu_m'} = \frac{\sqrt{(1-[w']^2/c^2)}}{1 + w'/u_m''},\quad \frac{\nu_0''}{\nu_0} = \frac{\sqrt{(1-w^2/c^2)}}{1 + w/u_0''}$$

Now the rear wall of the container is at rest in S'' so that the light entering it is unchanged in frequency for an observer at rest in S'':

$$\nu_0'' = \nu_m'' \qquad (6)$$

Hence to first order in v/c:

$$\frac{\nu_m'}{\nu_0} = \frac{\nu_0''/\nu_0}{\nu_m''/\nu_m'} = 1 + \frac{w'}{u_m''} - \frac{w}{u_0''}$$

The last two terms are correction terms and need be evaluated only to zero order, so that:

$$w' \doteqdot w - v,\quad u_m'' = c/n(\nu_0'') \doteqdot c/n(\nu_0)$$

Noting that $u_0'' = c$, one finds that equation (4) is valid, provided:

$$g = \frac{w + (v - w)n(\nu_0)}{c} \qquad (7)$$

Combination of (5) and (7) yields an expression for the drag coefficient. One notes that $w = 0$ corresponds to the case of water flowing through a tube, while $w = v$ corresponds to the moving block of glass in accordance with (2). One can say qualitatively: The effect of dispersion on the drag coefficient goes up with the disparity between the velocity of the medium and the velocity of the rear face of its container.

I am indebted to Prof. J. L. Synge for helpful correspondence.

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[1] Synge, J. L., *Relativity: The Special Theory*, 142 (North-Holland: Amsterdam, 1956).

RADIATION CHEMISTRY

Photolysis of Ethyl Iodide Solutions

ONE of the major products in alkyl iodide photolysis in various solvents is iodine, formed by combination of iodine atoms produced in the primary dissociation. Although the reaction is well known, quantum yields reported for iodine vary from zero to one[1-6]. In part, the scatter in the data from different laboratories is caused by the presence of undetermined amounts of oxygen. Hacobian and Iredale[6] pointed out the difficulty of complete removal of oxygen from these solutions in organic solvents, and found that the iodine yield decreased from 0·5 to less than 0·05 as the solution was degassed. Earlier work[1,5] indicated a decrease of about a factor of two in iodine-yield on removal of oxygen.

The present research started with the premise that complete removal of oxygen from a hydrocarbon solvent is best accomplished by chemical reaction. Alkaline pyrogallol solutions were equilibrated with the solute, ethyl iodide, and the solvent, *n*-hexane. After separation of the layers, each component was subjected to exhaustive vacuum distillation and outgassing. The reagents could then be distilled from storage into a 1-cm. path-length silica vessel and sealed off. Photolyses were carried out in the full arc of a 250-watt B.T.H. mercury arc lamp, which gave a weighted mean absorbed wave-length of about 3100 Å. Absorbed intensities during photolysis were obtained by ferrioxalate actinometry[7]. The concentration of iodine produced was measured directly in the reaction cell at 5250 Å.

In the absence of oxygen, the initial iodine quantum yield was 0·017, which agrees qualitatively with the value of < 0·05 found by Hacobian and Iredale. However, it was observed that as iodine accumulated in the solution the quantum yield decreased considerably. The effect can be interpreted on the basis of the reaction[8] :

$$C_2H_5 + I_2 = C_2H_5I + I \qquad (1)$$

As iodine accumulates in the system, reaction (1) increases in importance compared with the dimerization and disproportionation of ethyl radicals. In the limit of high-conversions, iodine reaches a steady-state concentration and the quantum yield of iodine formation approaches zero.

After equilibration with air the initial iodine quantum yield was higher by a factor of two than that in the complete absence of oxygen. It is recognized that oxygen effects the removal of ethyl radicals from the system, or, at least, prevents them from reacting with iodine atoms. Thus the quantum yield is generally higher in the presence of oxygen than in its absence because secondary recombination is largely prevented. The probable mechanism can be represented by the following secondary reactions, following dissociation into ethyl radicals and iodine atoms :

$$2C_2H_5 = C_4H_{10} \text{ or } C_2H_4 + C_2H_6 \qquad (2)$$

$$C_2H_5 + I = C_2H_5I \qquad (3)$$

$$2I = I_2 \qquad (4)$$

$$C_2H_5 + O_2 = \text{products} \qquad (5)$$

(It must be kept in mind, however, that only the rate formation of iodine molecules was followed in the work described.) In the absence of oxygen, reactions 2, 3, and 4 are the reactions involved in the formation of iodine, while in the presence of oxygen 4 and 5 are the only reactions taking part. Then it can be shown that the ratio of iodine quantum yield with oxygen present to that with oxygen absent is given by :

$$R = 1 + k_3/2(k_2k_4)^{1/2}$$

If the values of the rate constants k_2, k_3 and k_4 in solution are determined by statistics, that is, $k_2 = k_4 = \frac{1}{2}k_3$, R would have the value 2, in agreement with our experiments. Hacobian and Iredale found experimentally a value of R in excess of 10, which would require $k_3^2 > 300k_2k_4$. However, there is no reason to suspect reactions 2 and 4 to be so inefficient in solution compared with reaction 3.

This ratio of rate constants, based on the same mechanism, gives for the initial iodine quantum yield in the absence of oxygen :

$$\Phi_{I_2} = \frac{\varphi}{2 + k_3/(k_2k_4)^{1/2}}$$

where φ represents the quantum yield of dissociation into fragments free of the solvent cage. The value of φ determined by these experiments is 0·07. However, the possible presence of a scavenger capable of reaction with iodine atoms was not eliminated. Although this would not affect qualitatively the conclusions regarding the decrease of Φ_{I_2} with increasing conversion or its increase in the presence of oxygen, the absolute value of Φ_{I_2} could be in error. For this reason, aside from the technique of rigorous purification of solvent employed by Lampe and Noyes[8], it would be most desirable to remove oxygen with an inorganic reducing agent to avoid all possibility of introducing impurities which may act as inhibitors.

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Tritium as a Source of Radiation in Electron Spin Resonance Studies

A COMMON difficulty encountered in electron spin resonance investigations of materials irradiated by high-energy radiations (cobalt-60 γ-rays, fast electrons, etc.) is the appearance of the electron spin resonance spectrum of the irradiated glass (or quartz) tubes containing the samples.

The spectrum often becomes interpretable only after either 'bleaching' the glass or transferring the sample to a non-irradiated tube. Both procedures may be particularly awkward in experiments involving stabilization of free radicals at low temperatures.

It was expected that the above difficulties might be avoided by using internally emitted tritium β-particles, which, owing to their very low energies (< 18 keV.) and ranges (about 1μ in glass and most solid media), should leave the glass of the sample tube practically unaffected even for relatively high doses of radiation absorbed in the sample.

In order to test this conception, solutions of tritium oxide in water, deuterium oxide and water + deuterium oxide mixtures with activities 100 mc./ml. and 1 c./ml. were frozen down in glass tubes in liquid nitrogen. The electron spin resonance spectra of the samples irradiated in this way were then examined at liquid-nitrogen temperature.

After applying doses of radiation up to 5×10^{20} eV./ml., no effects of the irradiated glass on electron spin resonance spectra were observed.

The spectra of tritium β-irradiated water and deuterium oxide ice consist of one doublet and one triplet, respectively, and are interpreted as due to OH and OD radicals.

The corresponding g values, separation and line-widths are almost identical with those reported for cobalt-60 γ-irradiated ice[1]. The yields of OH and OD formation [(G(OH), G(OD)], and the saturation concentrations of both radicals seem to be lower than for γ-rays. This difference may be due to the higher linear energy-transfer of tritium β-particles.

In the presence of perchloric acid in tritiated water and deuterium oxide ice, hydrogen atoms and deuterium atoms are readily formed as in the experiments by Livingston and Weinberger[2], using cobalt-60 γ-rays.

The spectrum of a tritiated mixture of water, deuterium oxide and perchloric acid indicates the presence of all the radicals concerned, that is, H, OH, D and OD, and enables one to compare directly their rates of formation and stabilities.

Full details and further discussion of these experiments will be published elsewhere.

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CHEMISTRY

Nature of the Metallic Orbital

IN the course of the development of the resonating-valence-bond theory of metals[1] it became evident[2] that the characteristic structural feature of metals is the possession by each of many atoms in the metal of an extra orbital (the metallic orbital) in addition to the orbitals normally occupied by electrons. This metallic orbital permits the unsynchronized resonance of covalent bonds from one interatomic position to another by the jump of an electron from one atom to an adjacent atom without the need for an opposite jump to retain exact electric neutrality. The metal can thus be described as containing atoms with formal charges + 1 and − 1 as well as zero (larger formal charges are ruled out by the electroneutrality principle[3]).

A puzzling feature of the theory has been the fractional number, 0·72, of metallic orbitals per atom that is found by analysis of the values of the saturation ferromagnetic moments of the metals of the iron group and their alloys[2]. A simple derivation of an approximate value of this number, reported here, has now been discovered.

Let us consider[4] a metal such as lithium, with one valence electron per atom. The bonds occupy principally the positions between each atom and its eight nearest neighbours in the body-centred cubic crystal. The probability of a bond in a position is 1/8, and the probability of a vacancy is 7/8; it is easily seen that the relative probabilities of Li^+, Li and Li^- (with 0, 1 and 2 bonds, respectively) are in the ratios 7 : 8 : 4, or, with Li^+ and Li^- made equal by taking the geometric mean, 0·285 : 0·430 : 0·285. For other metals similar calculations give ratios lying between this limit and 1/3 : 1/3 : 1/3.

Atoms M^+ and M need to have a metallic orbital in order to be able to accept an additional bond; M^-, however, does not need a metallic orbital, because it is prevented by the unfavourable energy term of M^- from accepting another bond. Hence the number of metallic orbitals per atom is expected to be the sum of the probabilities of M^+ and M. This sum, as given by the above calculation, lies between 0·715 and 0·67. The experimental value, 0·72, is close to one end of this range; it corresponds with 28 per cent M^+ and M^-. It is, of course, likely that the probabilities of M^+ and M^- relative to M are decreased somewhat by the energy of separation of charge.

As an example, we may consider white tin, for which the observed interatomic distances indicate the valence to be about 2·64. There are four outer orbitals ($5s5p^3$), and Sn^+, Sn and Sn^- have 3, 4 and 5 outer electrons, respectively. The first two must have a metallic orbital; hence their valencies are 3 for Sn^+ (spp), 2 for Sn (s^2pp) and 3 for Sn^- (s^2ppp), with weighted average 2·56, in agreement with the value given by the interatomic distances.

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Nature of the Reactions involved in the Pyrolysis of *n*-Butane inhibited by Propylene

IN order to explain the observed features of the pyrolysis of paraffin hydrocarbons in the presence of such rate-inhibitors as nitric oxide, propylene and *iso*butene, it has been suggested[1] that the uninhibited reaction consists of two independent, concurrent mechanisms. The first is assumed to be an unbranched free-radical chain process, the second, a molecular elimination process. It is assumed[1] that the former can be entirely suppressed by the addition of sufficient inhibitor. In the pyrolysis of *n*-butane, for example, the reactions responsible for the production of the major products through the free-radical mechanism are most likely to be :

$$C_4H_9^{\cdot} \longrightarrow CH_3^{\cdot} + C_3H_6 \qquad (1)$$

$$C_4H_9^{\cdot} \longrightarrow C_2H_5^{\cdot} + C_2H_4 \qquad (2)$$

$$CH_3^{\cdot} + C_4H_{10} \longrightarrow CH_4 + C_4H_9^{\cdot} \qquad (3)$$

$$C_2H_5^{\cdot} + C_4H_{10} \longrightarrow C_2H_6 + C_4H_9^{\cdot} \qquad (4)$$

and, in the supposedly molecular portion of the reaction :

$$C_4H_{10} \longrightarrow CH_4 + C_3H_6 \qquad (I)$$

$$C_4H_{10} \longrightarrow C_2H_6 + C_2H_4 \qquad (II)$$

Both mechanisms predict equal yields of ethylene and ethane.

Alternative interpretations of the experimental evidence[2,3] discount the possibility of the participation of molecular processes and attribute the completely suppressed reaction to some form of modified radical chain.

We have used the technique of high-speed gas-liquid chromatography[4] in conjunction with a high-sensitivity flame-ionization detector to study both the uninhibited pyrolysis of *n*-butane and the reaction in the presence of varying amounts of propylene. The identity of the products was unchanged under all conditions, and the distribution of the major products, as reflected in the ratio [methane/(ethane + ethylene)], was virtually independent of the pressure of propylene. In the uninhibited reaction the ratio C_2H_4/C_2H_6 ($= \alpha$) was found to be consistently greater than unity, being commonly approximately 2, while values as large as 4 have been obtained.

Some results obtained in the investigation of the pyrolysis at 778·7° K., under a pressure of 19·2 mm. mercury, of *n*-butane in the presence of various pressures of propylene are summarized in Fig. 1. It is seen that the overall rate of reaction is reduced by a factor of about three on increasing the pressure of propylene to 6 mm. The value of α, however, increases linearly through this region, and beyond, rising from 1·9 to 2·5 as the pressure of propylene changes from 0 to 30 mm.

If now it is assumed that the fully suppressed reaction [$C_3H_6 > 6$ mm.] is completely molecular in character, it follows that the intercept on the ordinate of the plot of α against pressure of propylene represents the value of α pertaining to any molecular processes occurring in the uninhibited reaction. This value is found to be identical with that determined in the experiment carried out in the absence of propylene. This identity is of considerable importance since it requires that, if the residual reaction is entirely molecular, either (*a*) the uninhibited reaction is entirely molecular, or (*b*) if radical and molecular processes occur concurrently in the uninhibited reaction, then :

$$\alpha_{\text{observed}} = \alpha_{\text{radical}} = \alpha_{\text{molecular}} \qquad (A)$$

The possibility that (*a*) is true can be immediately discounted.

Since results similar to those shown in and deduced from Fig. 1 were obtained in all the experiments conducted over the usual ranges of temperature and initial pressure of *n*-butane, it is evident that, if (*b*) is true, relation (*A*) must hold in all such conditions. That is, whatever mechanisms give rise to $\alpha > 1$, both radical and molecular, must have identical kinetic characteristics.

The characteristics associated with the formation of this 'excess ethylene' [$= C_2H_4 - C_2H_6$] in the uninhibited reaction are :

(i) α remains constant throughout at least the initial 5 per cent of any given reaction.

(ii) The excess of ethylene is formed at the expense of the ethane, since the C_2 hydrocarbons form a constant proportion of the product at any given temperature.

(iii) The variation with temperature of the function :

$$\alpha - 1 = \frac{[C_2H_4] - [C_2H_6]}{[C_2H_6]}$$

at any given pressure of *n*-butane is associated with an activation energy of (28·6 ± 2·5) k.cal./mole.

(iv) The total order of the reaction forming the excess of ethylene is less than that of the reaction forming the ethane, and varies with total pressure.

The inclusion of the reaction :

$$C_2H_5^{\bullet} \longrightarrow C_2H_4 + H^{\bullet} \qquad (5)$$

in the free-radical mechanism adequately accounts for the findings summarized in (i) to (iv). On the other hand, while several molecular reactions which could give rise to $\alpha > 1$ can be postulated, it seems impossible to envisage any counterpart of reaction (5) which, in combination with (I) and (II), would be compatible with relation (*A*) and the restrictions imposed by (i)–(iv). Thus, possibility (*b*) must be eliminated.

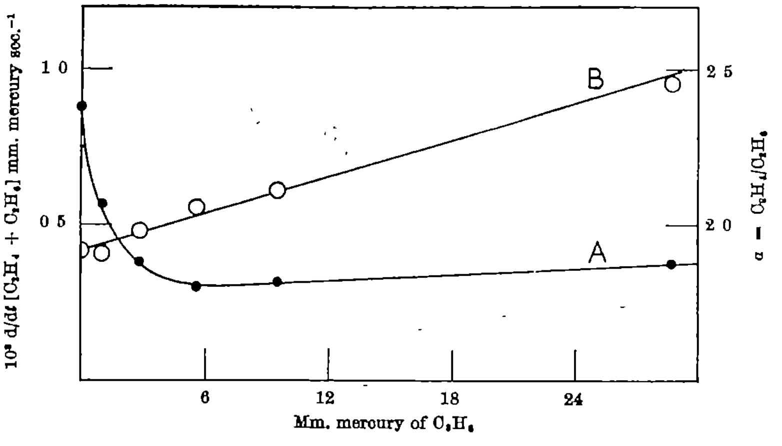

Fig. 1. Effect of the addition of propylene on the pyrolysis of 19·2 mm. mercury of *n*-butane at 778·7° K. Plots show the dependence on pressure of propylene of (*A*) the initial rate of formation of the total C_2 fraction and (*B*) the ratio $\alpha = C_2H_4/C_2H_6$

Since, therefore, molecular processes appear to play no part in the completely inhibited reaction, it is evident that both the uninhibited and inhibited reactions must be regarded as entirely free-radical in character. This conclusion is in harmony with the experimental findings which, for the completely suppressed reaction, are then best described in terms of a modified free-radical chain reaction propagated by radicals of low reactivity[3].

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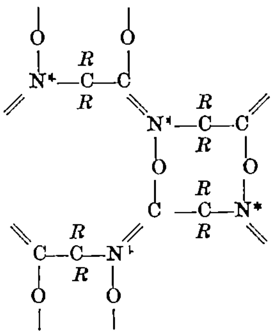
Possibility of Ionic Dissociation at the Peptide Linkage

THE need for a cyclic structure for certain proteins (for example, serum globulins) has been emphasized in a series of papers by Wrinch[1,2]. However, the hexacyclic carbon–nitrogen ring structure which she proposes has not been generally accepted, due partly to steric objections. It is believed at present that two-dimensional protein structures are formed by hydrogen bonding between polypeptide chains. In the present communication a new oxygen cross-bonded structure is suggested. It is characterized by ionic dissociation, and a double bond, at the peptide linkage. The structure is:



where N* is either N^- or $(HNH)^+$ depending on whether the dissociation:

$$NH \rightarrow N^- + H^+ \qquad \text{(acidic)}$$

or

$$NH + H^+ \rightarrow NH_2^+ \qquad \text{(basic)}$$

occurs. The amino-acid residues (*R* groups) and the H ions of $(HNH)^+$ groups lie perpendicularly to the plane of the structure.

The reason why both acidic and basic dissociations are thought to be possible is as follows. In the above structure, N atoms complete their $n = 2$ shell octet by sharing 4 of their 5 valence electrons in covalent bonds with neighbouring atoms. The fifth valence electron is therefore forced up into a 3*s* level where it will behave very much like the 3*s* valence electron of sodium. One knows that sodium hydride dissociates into Na^+ and H^- ions (the latter combining with H^+ ions of water to give H_2) and consequently one might expect that NH would dissociate similarly. This is essentially the basic dissociation shown above. However, the 3*s* electron of the N will be more tightly bound than the 3*s* electron of Na when the adjacent amino-acid residues are electrophilic (electron deficient), because then the covalent bonds to the N atom will tend to be polar, causing the N charge to exceed by a slight amount one electron charge. In this case acidic dissociation of NH may be expected. The dependence of the type of dissociation on the electrophilic or nucleophilic character of adjacent amino-acid residues means that the genetical code of a protein residing in its sequence of amino-acids can be transferred to a sequence of ionic charges at the peptide linkages.

The delicate balance between acidic and basic dissociation at the peptide linkage will mean that the free energy of dissociation is exceptionally small. One therefore expects proteins to be precipitated by slight changes to the solvent. It is well known that slight changes of *p*H and reagents such as methanol do, unaccountably, precipitate protein. One may expect also a tendency of positively charged ions of the correct electronegativity to complex with N^- ions at the peptide linkage. This may explain the denaturation of proteins by heavy ions in solution, and possibly also the different response of biological organisms to the chemically similar Na^+ and K^+ ions and the chemically similar Ca^{2+} and Mg^{2+} ions.

A definite need to invoke new dissociations has been found in the case of the proteins of tobacco mosaic virus[3] and in the case of silk fibroins[4]. The net charge of these proteins indicated by electrophoretic measurements differs considerably from that deduced from chemical analysis (for the same *p*H). Furthermore the *p*H of minimum movement under an electric field (the true isoelectric point by definition) differs from the *p*H of minimum combination with acid or alkali (often termed the isoionic point).

Preliminary experiments I have made show that ultra-violet irradiation can alter the net charge of serum protein. Part of a protein front moving along an electrophoretic strip in a buffer of *p*H 8·6 was made to pass through a narrow band of ultra-violet radiation. In the case of negatively charged protein (albumins) the ultra-violet irradiation produced an acceleration, while in the case of positively charged protein (γ-globulins) a retardation was noted. It would seem that the ultra-violet irradiation tended to increase the net negative charge of the protein by destroying positive charges. Since it is reasonable to assume that the ultra-violet irradiation is absorbed at a double bond, this appears to constitute evidence for ionic dissociation at the peptide linkage.

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BIOCHEMISTRY

Induced Protein Synthesis

A POSSIBLE relationship between the synthesis of induced enzymes and the formation of antibodies was suggested in 1900 by Dienert[1]. Compelling arguments[2] have since accumulated to support this contention, but corroborative experimental evidence has not been forthcoming until recently. This laboratory has been engaged in obtaining such evidence by an investigation of the specificities of antibodies and enzymes induced by the same determinant group.

Known inducers for specific enzyme systems were coupled to bovine serum albumin with the determinants held in covalent linkage putative to the bond which might exist in comparable inducer-macromolecular complexes in microbial systems. The specificities of rabbit antibodies to these conjugated proteins were studied by hapten-inhibition techniques, on the premise that a similarity in the mode of formation of antibodies and induced enzymes might be reflected in analogous conformational and stereochemical requirements at their respective combining sites. The antibody specificities determined in this manner were then compared with previously reported specificities for the haptens acting as inducers or substrates for the corresponding induced enzyme systems. A good correlation was observed between the specificity of rabbit antibodies to protocatechuoyl-bovine serum albumin and the induction of protocatechuic acid oxidase in *Neurospora*[3]; between the specificity of antibody to β-D-galactosido-phenylazo-bovine serum albumin and the β-D-galactosidase enzyme system of *E. coli* and *B. megaterium*[3,4]; and between the inducible β-D-glucosidase of *Rhodotorula minute*, the constitutive β-D-glucosidase from *Stachybotrys atra*, and antibodies formed in response to β-D-glucosido-phenylazo-bovine serum albumin[5]. The results also suggested[3] that the inducible mechanism (permease)[6] for the transport of β-D-galactosides into *E. coli* may follow the specificity requirements of other β-D-galactoside accommodating systems.

The similarity between the specificities of antibodies to β-D-galactosido-phenylazo-bovine serum albumin and the corresponding inducible enzyme in *E. coli* was further demonstrated experimentally, as shown in Table 1. The results of a typical series of experiments employing a 1 : 5 : 16 ratio (based on nitrogen) of enzyme, antigen, and antibody are presented. Incubation of the synthetic conjugated antigen β-D-galactosido-phenylazo (Gal)-bovine serum albumin with an enzyme extract from *E. coli*, prior to addition of antibody, inhibited hydrolysis of the chromogenic substrate, *o*-nitrophenyl-β-D-galactoside. In other experiments the concentration of antigen was reduced tenfold, with other concentrations held equal, and pre-incubation of Gal-bovine serum albumin with enzyme still completely inhibited hydrolysis of *o*-nitrophenyl-β-D-galactoside. Galactosyl groups were not hydrolysed from Gal-bovine serum albumin by β-D-galactosidase. This indicates that the binding sites of the enzyme were specifically occupied, while the catalytic site may have been sterically hindered by the bulk of the conjugated antigen. Simultaneous addition of the antibody to enzyme and Gal-bovine serum albumin did not affect enzyme action. It appears, therefore, that the binding sites of the enzyme and the antibody can compete for the specific polyvalent non-substrate. The enzyme-Gal-bovine serum albumin complex, once formed, was not dissociated by antibody. With simultaneous additions, the antibody reacted with antigen more rapidly, and possibly more firmly, than did the enzyme. It might be possible to reverse inhibition of hydrolysis of *o*-nitrophenyl-β-D-galactoside by enzyme with Gal-bovine serum albumin provided that sufficiently high concentrations of substrate are added. It will be of interest to study the kinetics of competition of enzyme and antibody for antigen. It should also be noted that antibody had no catalytic effect on hydrolysis of substrate, although hapten-inhibition tests[4] demonstrated strong specific binding of this substrate at the antibody combining site.

Table 1. COMPETITION BETWEEN *E. coli* β-D-GALACTOSIDASE AND RABBIT ANTI-β-D-GALACTOSIDO-ANTIBODY FOR β-D-GALACTOSYL-BOVINE SERUM ALBUMIN

Series	First additions	Second additions	Third additions	O.D. 420 mμ (× 10³)
1	E+BSA*	—	ONPG	288
2	—	E+BSA	ONPG	308
3	Gal-BSA+E	Ab	ONPG	88
4	—	E+Gal-BSA+Ab	ONPG	298
5	Gal-BSA+Ab	E	ONPG	314
6	—	Ab+ONPG	E	305
7	—	Ab+E	ONPG	321
8	—	Ab+ONPG	BSA	32
9	Gal-BSA+E	—	—	†

* BSA added as inert protein to prevent enzyme denaturation.

† 8 mgm. Gal-BSA and 300 μgm. N enzyme protein. No release of galactosyl groups as determined (*a*) by inability to detect free galactose with paper chromatography, and (*b*) no increase in sugar in supernatant, using anthrone method (ref. 14), after precipitation of Gal-BSA and enzyme with trichloracetic acid.

Enzyme (E), 0·1 ml. fraction *d* (Wallenfels *et al.*, *Biochem. Z.*, 331, 459; 1959) from induced *E. coli* containing 0·42 μgm. N enzyme protein; antigen (Gal-BSA), β-D-galactosido-phenylazo-bovine serum albumin (ref. 4), 0·2 ml. containing 2 μgm. N; antibody (Ab), 0·2 ml. absorbed rabbit antiserum (refs. 3 and 4) with 7·4 μgm. antibody N; bovine serum albumin (BSA), 0·3 ml. saline solution with 2·1 mgm. protein; *o*-nitrophenyl-β-D-galactoside (ONPG), 1·0 ml. of 3×10^{-3} *M* in phosphate buffer, *p*H 7·0. Tubes incubated at 30° C. 30 min. elapsed between first and second and between second and third additions. 10 min. after last addition, 2·0 ml. 0·1 *M* sodium carbonate added for colour development. Final volumes adjusted to 3·5 ml. as necessary. Optical densities corrected for protein absorption at 420 mμ.

Monod has stated[2] that the number of antibodies which may be produced by an organism of one genotype is infinite, whereas the numbers of enzymes inducible in one particular species of bacterium is quite limited. This conjecture may reflect the use of the extremely non-versatile organism *E. coli* for most induction studies. Consideration of the Pseudomonadaceae, for example, shows that the genotype of these micro-organisms allows for an almost unlimited number of enzyme inductions. From the classic example of Den Dooren de Jong[7] with *Pseudomonas putida* to the sequential induction experiments of Stanier[8] and of Dagley and co-workers[9], it is obvious that these microbes are capable of forming literally hundreds of enzymes which finally integrate fragments of unusual substrates into normal metabolic pathways. It is difficult to visualize "repressor substances"[10] being continuously synthesized for the myriad of enzymes which are inherent in the genome of Pseudomonads. Again, species differences in the induction of antibodies are also known. The most familiar example is the difference between rabbits and human beings in their ability to produce antibodies to purified polysaccharides. If one were to rely on the rabbit alone as a test animal, it might be concluded erroneously that purified polysaccharides are non-antigenic.

The formation of induced enzymes and antibodies has two requisites ; (*a*) the inducible organism must have the genetic potential to produce the specifically directed protein ; (*b*) the inducer must be present. Benzer[11] has demonstrated that the inducer does not act by selection in microbial enzyme induction. On the other hand, the Burnet–Lederberg "clonal-selection" hypothesis[12,13], which has been advanced to explain the phenomena of "self-recognition" and "actively-acquired tolerance", postulates that the antigen causes existing cells, which are capable of forming the specific antibody, to proliferate.

The observed parallelism presented here between the specificities of microbial enzymes and mammalian antibodies formed in response to the same determinant group suggests that, regardless of the mechanism, directed protein synthesis depends on the genetic endowment of a particular species to form a peptide chain the ultimate three-dimensional conformation of which is determined by the molecular configuration of the inducer.

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Amino-Acid Patterns in Vitamin A-deficient Rats

VITAMIN A deficiency seems to cause negative nitrogen balance in young rats[1]. In a preliminary report, Abdulnabi and Bieri[2] indicated that in vitamin A-deficient as compared to pair-fed normal rats, the tyrosine- and phenylalanine-levels in the testes are increased and the threonine concentration of the liver shows a decrease. These results were obtained with microbiological assays, since paper chromatography failed to reveal any significant differences. We have compared the free amino-acid composition in blood, liver and kidneys of vitamin A-deficient and pair-fed normal rats by paper chromatography. Our results confirm the earlier observations of Abdulnabi and Bieri[2] and show significant increase in the phenylalanine- and tyrosine-levels, and a simultaneous significant decrease in the threonine values in all the three tissues of the deficient rats.

Weanling male albino rats of this Institute strain weighing 30–40 gm. were used in our experiment, and were kept on a vitamin A-free diet[3]. The control animals were given the same diet, but received, in addition, 150 µgm. of vitamin A once a week. The animals on the vitamin A-free diet alone stopped growing after about four weeks and began to show signs of A-deficiency. At this stage the control animals were pair-fed with the deficient ones. At the end of the sixth week, when severe symptoms of A-deficiency, as judged by acute anorexia and loss of weight, developed, the animals of both groups were killed. Blood was collected by heart puncture and liver and kidneys were excised. All the tissues were immediately cooled to 0°.

The free amino-acids of blood were extracted by the method of Giri *et al.*[4], and those of liver and kidneys were obtained by extraction with 80 per cent ethanol. The amino-acids were separated by circular paper chromatography on Whatman No. 1 filter paper and estimated colorimetrically[5]. Cystine, lysine, histidine, arginine, alanine, tyrosine, phenylalanine and the leucines were separated in a system[5] of *n*-butanol/water/acetic acid (40 : 50 : 10). The chromatogram was irrigated three times in order to ensure good separation. Valine and methionine were separated[6] in butanol/acetic acid/water (40 : 5 : 7). Triple irrigation was resorted to here also. Aspartic acid, glutamic acid, serine, glycine and threonine were separated in a phenol/water/*iso*propanol (70 : 25 : 5) system in an atmosphere saturated with 0·3 per cent ammonia (two runs)[7].

Chromatograms with the butanol/acetic acid/water systems were run in a thermostatically controlled room maintained at 18°. Standard amino-acids (10 µgm.) were always spotted along with the test materials. These served not only as reference, but also for final estimation of the unknown samples. It has been our experience that these procedures give consistent values.

The average patterns of free amino-acid in blood, liver and kidneys of seven groups of rats, each group consisting of one deficient and one normal pair-fed, are summarized in Table 1. Twelve out of the fifteen amino-acids estimated did not show any significant differences, whereas phenylalanine and tyrosine were markedly higher and threonine was consistently lower in all the three tissues of the deficient animals. The *P* values obtained by statistical analyses were less than 0·001 for these three amino-acids in all the tissues. It should, however, be mentioned here that Abdulnabi and Bieri[2] had pointed out significant depression of threonine in the liver and increases in phenylalanine and tyrosine values of testes only. Our results show more consistent changes in the three amino-acids in all the tissues examined.

Ershoff[8] had suggested that vitamin A may have some role in the proper functioning of the adrenal gland, and Lowe *et al.*[9] have actually shown histochemical changes in the same glands during vitamin A-deficiency. It has since been reported that the synthesis of glucocorticosteroid hormones is impaired in vitamin A-deficient animals[10]. This might be due to the failure of hydroxylation of the steroids. Production of adrenaline would require the introduction of three hydroxyl groups into the phenylalanine molecule. It is, thus, tempting to suggest

Table 1. Amino-acid Patterns in the Tissues of Vitamin A-deficient and Pair-fed Rats

Amino-acids	Average values for seven groups of rats					
	Blood (μgm./ml.)		Liver (μgm./gm.)		Kidneys (μgm./gm.)	
	A	*B*	*A*	*B*	*A*	*B*
Aspartic acid	52 ± 2 7*	51 6 ± 1·7	353 ± 13·6	355 ± 10·5	995 ± 10·2	989 ± 12·7
Glutamic acid	29 ± 1·7	28 2 ± 2 3	356 ± 12 4	358 ± 10·9	249 ± 14·6	240 ± 10·4
Serine	62 8 ± 4 4	60·0 ± 5·4	504 ± 4 2	506 ± 4·3	1,094 ± 12·0	1,108 ± 11·3
Glycine	30 4 ± 2 6	31·7 ± 2·5	498 ± 6 2	498 ± 5·7	751 ± 8·8	749 ± 10·4
Cystine	147·7 ± 9·5	147 4 ± 10 2	195 ± 4·3	197 ± 2·6	186 ± 5·7	182 ± 4·7
Lysine	77 4 ± 4·7	79·0 ± 3·5	574 ± 13·3	579 ± 13 0	723 ± 12·2	723 ± 10·5
Histidine	52·1 ± 3 0	53·1 ± 3·1	244 ± 6·4	243 ± 4 2	567 ± 14·5	563 ± 10·5
Arginine	113 ± 4·2	110·7 ± 2·4	440 ± 5·7	439 ± 7·3	1,770 ± 17·8	1,765 ± 10·7
Alanine	49 ± 1·1	50·2 ± 1 6	1,175 ± 15 9	1,168 ± 14 7	1,756 ± 12·5	1,753 ± 13·6
Methionine	17 7 ± 1·4	18·5 ± 1·1	104 ± 2·6	106 ± 2 3	96 ± 1·3	93 ± 2 3
Valine	28 9 ± 1 0	28 8 ± 1 1	396·4 ± 4·5	395 ± 4 8	253 ± 3·1	256 ± 4·0
Leucines	16·4 ± 2 9	16 2 ± 0·1	534 0 ± 5 4	536 ± 5·0	942 ± 7 7	939 ± 7·4
Threonine	40·0 ± 4·3	64·0 ± 5·4	1,469 ± 15·9	2,089 ± 15·9	2,453 ± 55·9	3,941 ± 58 9
Tyrosine	104 5 ± 3·5	60 8 ± 3·3	1,032 ± 22 3	808 ± 10·7	909 ± 5·5	699 ± 10 7
Phenylalanine	88·6 ± 4 2	49 8 ± 4·6	1,469 ± 15·0	965 ± 6·4	1,894 ± 10 8	1,579 ± 10·5

* Standard error of mean. *A*, deficient rats. *B*, pair-fed normals.

that the accumulation of phenylalanine and tyrosine in the tissues of the vitamin A-deficient rats might be due to the impairment, in the damaged adrenal glands, of the hydroxylation reactions that are required for the production of adrenaline from phenylalanine.

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Enzymatic Dephosphorylation of Adenosine Tetraphosphate

Adenosine tetraphosphate was discovered as an impurity in some commercial preparations of adenosine triphosphate[1,2] where its concentration may exceed 10 per cent of the total adenine[3]. It was also found in horse muscle[4] and possibly in rat liver[5].

The chemical properties of this nucleotide indicate that its structure is that of a straight chain of four phosphates attached to the carbon 5′ of adenosine:

adenine—ribose—P—P—P—P

Three of its four phosphates are acid labile[1,6]; the compound has two neighbouring hydroxyl groups[4,7], three primary phosphate groups (unpublished work) and only one secondary phosphate group[7]. In the following experiments the new nucleotide was dephosphorylated in the presence of specific enzymes.

A *b*-nucleotidase preparation was obtained from potato juice and purified by precipitation with alcohol and chloroform, adsorption on alumina gel and fractionation with ammonium sulphate. When tested at *p*H 9·5 and in the presence of 8 m*M* magnesium chloride, the isomer 3′ (or *b*) of adenylic acid was the only substrate hydrolysed, whereas the isomers 2′ (or *a*) and 5′, adenosine diphosphate, triphosphate and tetraphosphate were not. This indicated that adenosine tetraphosphate has no phosphate group attached to the carbon 3′.

An apyrase was also obtained from an acetone powder of potato juice and further purified by ammonium sulphate fractionation at *p*H 4·5. When tested at 40° C. and *p*H 6·5 in the presence of 3 m*M* calcium chloride and of 0·01 per cent serum albumin, the preparation liberated about 345 μgm. of orthophosphate per μgm. of enzyme preparation per hour, with 7·5 m*M* adenosine triphosphate as substrate. Compared with this figure taken as 100, adenosine diphosphate and tetraphosphate were hydrolysed more slowly (28 and 12 per cent respectively). In the presence of higher enzyme concentrations, adenylic acids (isomers 3′ and 5′ only), inorganic pyrophosphate and β-glycerophosphate were very slowly hydrolysed (0·2–0·5 per cent of the rate measured with adenosine triphosphate).

When a relatively large amount of this enzyme preparation was incubated with a small amount of adenosine tetraphosphate, more than three phosphates could be liberated per mole of adenine. This indicates that the apyrase activity was followed by the nucleotidase activity of the preparation. Since the nucleotide has no phosphate group on carbon 3′, and since the enzyme preparation has no measurable 2′-nucleotidase activity, it follows that the 5′-nucleotidase activity must be responsible for the last phosphate liberated.

Finally, the adenylic acid produced during the enzymatic hydrolysis was chromatographed on 'Dowex-1 formate' according to Cohn[8] and shown to be the 5′-isomer exclusively. The same 5′-isomer had been identified enzymatically after a mild acid hydrolysis[4].

The evidence provided by the enzymatic dephosphorylation of adenosine tetraphosphate confirms the structure proposed on the basis of its chemical properties.

It is hoped to publish the details of these experiments in the *Bulletin de la Société de Chimie biologique*.

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Ninhydrin Assay in the Presence of Thiol Compounds

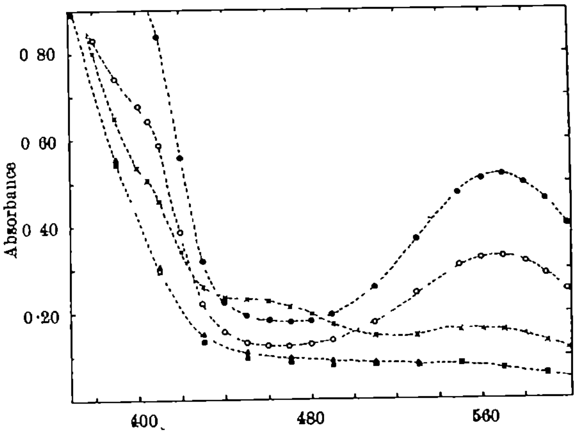

Fig. 1. Absorption spectra of the products produced by reaction with ninhydrin. ○, 0·10 μmole leucine; ×, 0·16 μmole cysteine; ●, 0·16 μmole cysteine + 4·0 μmoles NEM; △, ninhydrin reagent blank; □, ninhydrin reagent + 4·0 μmoles NEM

Moore and Stein[1] reported that the reaction of cysteine with ninhydrin is an exception to the general reaction of α-amino-acids with this reagent and the absorption curve of the reaction products is somewhat similar to that of proline and hydroxyproline (Fig. 1). They found that if the neutral protein hydrolysates were allowed to stand at room temperature for 24–28 hr. or if they were adjusted to *p*H 8 with sodium hydroxide and allowed to stand at room temperature for 4 hr. the cysteine–ninhydrin reaction product gave an absorption spectrum identical to that produced by cystine[1,2]. It is usual to include cysteine, mercaptoethanol, 2,3-dimercaptopropanol-1 or some other thiol compound as an activator for sulphydryl enzymes. When these are used for the activation of proteolytic enzymes such as ficin and papain the thiol compound ($10^{-2} - 10^{-3}$ *M* solutions) is not completely oxidized to the disulphide during the reaction procedure. The presence of the sulphydryl group then interferes with the usual ninhydrin determination as shown in Table 1 (col. 7). The presence of cysteine introduces two errors; first, during the enzymatic reaction it is partially oxidized to cystine (an unpredictable amount), and second, the cysteine remaining reacts with the ninhydrin reagent to give a red colour which interferes with the quantitative reaction of ninhydrin with the amino-groups. The presence of 0·1 μmole of mercaptoethanol in the modified ninhydrin reaction does not affect the quantitative determination of leucine (Table 1, col. 6), but, if the concentration of this reagent is increased to 10 μmole, leucine does not give a characteristic reaction product having a maximum absorption at 570μ but rather a red colour with an absorption maximum around 450 mμ is produced.

There would appear to be two solutions to this problem; first, the sulphydryl group could be oxidized to the disulphide in an alkaline medium, and second, the sulphydryl group could be blocked by reaction with a suitable reagent. Alkaline oxidation of the sulphydryl group of cysteine was found to proceed rather slowly at room temperature and did not give a quantitative conversion to cystine as measured by the ninhydrin method. Alkalis are known to have a destructive effect on certain amino-acids such as cysteine, cystine and arginine[3] and to cause racemization of amino-acids. N-ethylmaleimide reacts rapidly and quantitatively[4,5] with the sulphydryl group and has been used to prevent the oxidation of cysteine to cystine during separation by paper chromatography[6,7]. As shown by the results in Table 1 (compare cols. 2 and 3) leucine cannot be determined quantitatively in the presence of 4·0 μmoles of N-ethylmaleimide by the usual ninhydrin reagent[8]. If the amount of hydrindantin in the ninhydrin reagent is doubled, however, leucine (and other amino-acids tried) can be determined quantitatively in the presence of N-ethylmaleimide (Table 1, cols. 4 and 5). By the use of N-ethylmaleimide, a mixture of leucine and cysteine can be determined quantitatively (Table 1, col. 8) and the absorption spectrum of the product formed by the reaction of ninhydrin with the S-substituted cysteine is identical with that produced

Table 1. QUANTITATIVE DETERMINATION OF LEUCINE IN THE ABSENCE AND PRESENCE OF N-ETHYLMALEIMIDE (NEM), CYSTEINE AND MERCAPTOETHANOL (ME)

(1) Leucine (μmoles)	Optical density at 570 mμ						
	(2) Leucine*	(3) Leucine + NEM† (4·0 μmoles)	(4) Leucine‡	(5) Leucine + NEM (4·0 μmoles)	(6) Leucine + ME (0·1 μmole)	(7) Leucine + Cysteine (0·16 μmole)	(8) Leucine + Cysteine (0·16 μmole) NEM (4·0 μmoles)
0·050	0·124	0·056	0·119	0·123	0·126	0·221	0·569
0·100	0·255	0·139	0·263	0·261	0·265	0·352	0·692
0·200	0·514	0·289	0·527	0·531	0·540	0·611	0·951

* With the usual ninhydrin reagent (ref. 8). The sample was diluted to 2·0 ml. with water and then 1·0 ml. ninhydrin reagent added. After colour development, 5·0 ml. of 50 per cent (v/v) ethanol–water was added.

† The NEM is made up as a 2·02 *N* solution in 4·0 *N*, *p*H 5·5 acetate buffer and is added prior to addition of ninhydrin reagent.

‡ With ninhydrin reagent containing twice the usual amount of hydrindantin.

with leucine (Fig. 1). A study of the rate of reaction of N-ethylmaleimide with cysteine under the conditions employed here showed that the reaction goes to completion almost instantaneously. The N-ethylmaleimide is completely stable under acid conditions but should not be exposed, even momentarily, to alkaline conditions as it is converted to N-ethylmaleanic acid, which will then react with the ninhydrin reagent.

N-ethylmaleimide is used regularly in this laboratory to prevent the interference of sulphydryl groups with the ninhydrin assay of proteolytic activity and has been found to give very good results under a wide variety of conditions.

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Chemical Oxidation of Uridine Diphosphate-Glucose to Uridine Diphosphate-Glucuronic Acid

THE importance of uronic acids in carbohydrate metabolism has only recently begun to be elucidated. The metabolism of inositol and ascorbate is known to involve uronic acid intermediates[1,2], and nucleotide-bound glucuronic acid has been implicated in hyaluronic acid synthesis[3].

Uridine diphosphate (UDP)-glucuronic acid has been prepared chemically by Khorana and may also be produced enzymatically by the action of UDP-glucose dehydrogenase[4]. Both these preparations are relatively time-consuming and require the separation of the product from several contaminating reactants and side products.

Marsh has reported the oxidation of glucose-1-phosphate to glucuronic acid-1-phosphate[5], and this report describes a similar oxidation of UDP-glucose.

Powdered platinum catalyst was prepared in aqueous solution from Adam's platinous oxide catalyst by hydrogenation at atmospheric pressure. 100 mgm. of reduced catalyst were suspended in 10 c.c. of water and introduced into a narrow vessel similar to that described by Marsh. The cylindrical vessel and sintered glass disk were attached to a U-shaped tube and immersed in a constant temperature bath; oxygen was bubbled through the solution at a rate adequate to keep the heavy catalyst particles in suspension. The UDP-glucose solution (63 μmoles) was then added, and oxygenation was allowed to proceed for 10½ hr. at a temperature of 55°, the *p*H being maintained between 7 and 8 by addition of 1 per cent potassium carbonate. This contrasted with the oxidation of glucose-1-phosphate at 45°, at which temperature no UDP-glucuronic acid was produced. At the end of the reaction the catalyst was removed by filtration, washed, and the filtrate and washings assayed for uronic acid content[6].

In order to show that the uronic acid produced was still nucleotide-linked, an aliquot was spotted for paper electrophoresis in 0·1 *M* citrate-phosphate buffer, *p*H 2. The electrophoresis was carried out at 800 V. for 2 hr., and the two ultra-violet absorbing spots were eluted from the paper with water; one of them gave a positive carbazole test, uridine spectrum, and showed a 1 : 1 ratio of uronic acid to uridine based on a molar extinction coefficient for uridine of 10,000, the other material being unreacted UDP-glucose. The UDP-glucuronic acid could also be assayed by enzymatic transfer to *o*-aminophenol as described by Dutton and Storey, utilizing a fraction of rat liver homogenate sedimenting between 500*g* and 37,000*g* as the enzyme system[7].

Use of 5 per cent palladium on charcoal as catalyst and oxygenation of the reaction mixture for 7 hr. resulted in no increase in the amount of uronic acid present, indicating that no oxidation had occurred.

Table 1. VARIATION OF YIELD WITH TIME

Time (hr.)	Yield* (per cent)
5·0	12
5·5	24
10·5	39

* Based on recovered UDP-glucose.

On the basis of recovered UDP-glucose, the best yield under the conditions studied has been 39 per cent. It is felt that the ease of isolation and relatively short reaction time required make the reaction feasible for the production of UDP-glucuronic acid.

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Incorporation of [$1\text{-}^{14}C$]-*Iso*pentenyl Pyrophosphate into Polyisoprene

THE occurrence of 3-methyl-but-3-enyl-1-pyrophosphate (*iso*pentenyl pyrophosphate) as an intermediate in the enzymic synthesis of squalene from mevalonic acid, using yeast extracts, has been demonstrated by Bloch *et al.*[1,2], and by Lynen *et al.*[3]. The latter authors also quote an unpublished report by Arreguin that *iso*pentenyl pyrophosphate can be incorporated into the rubber of *Taraxacum kok-saghyz* latex. The *in vitro* conversion of mevalonic acid lactone into polyisoprene in the presence of natural rubber (*Hevea brasiliensis*) latex has been shown by Park and Bonner[4] and by Kekwick *et al.*[5]. In the experiments reported here it is demonstrated that *iso*pentenyl pyrophosphate, on incubation with fresh *Hevea brasiliensis* latex, is rapidly converted into high molecular weight polyisoprene with essentially 100 per cent efficiency.

Fresh latex was obtained from the young branches of two 18-months-old specimens of *Hevea brasiliensis* (Clone *P.B.* 86) growing in the tropical greenhouse attached to these laboratories. The [1-^{14}C]-*iso*pentenyl pyrophosphate (in amount too small for weighing) was dissolved in a suitable volume of 50 per cent aqueous alcohol to give the required radioactivity per unit volume. A known volume of this solution was used per incubation and evaporated to dryness under nitrogen in the incubation tube before addition of the fresh latex. The incubations were carried out under the conditions shown in Table 1. Each incubation tube was then spun about its axis to distribute the latex over the wall of the tube, and 10 drops of 5 per cent acetic acid in ethanol added. The thin film of coagulated rubber was covered with more coagulant for 5 min. and washed with water. It was next digested for 1 hr. at 100° C. with molar potassium hydroxide, washed with water, then with ethanol and finally extracted continuously with boiling redistilled ethanol for 16 hr. After evaporation of the residual alcohol on the rubber, 2 ml. of a 1 per cent solution of trichloracetic acid in dry toluene were added and the mixture stirred magnetically until the solution was homogeneous. The solution was then centrifuged at 10,000*g* and the supernatant quantitatively transferred to the counting vessel of an Ekco scintillation counter (type *N*664*A*). The residue left after centrifuging was negligibly small. The total count-rate of the toluene solution was determined by standard techniques, corrections being applied for the background count-rate and for counter efficiency, using an internal standard. The activity per unit volume of the original *iso*pentenyl pyrophosphate solution was determined similarly.

Table 1. INCORPORATION OF *iso*PENTENYL PYROPHOSPHATE INTO POLYISOPRENE

	1	2	3	4
Weight of latex (mgm.)	480	414	403	414
Total activity of *iso*pentenyl pyrophosphate added (c.p.m.)	6,360	16,380	6,360	16,380
Time of incubation	25 sec.	10 min.	30 min.	4 hr.
Temperature of incubation (° C.)	20	20	20	20
Percentage of initial radioactivity recovered in the rubber	24	102	98	100

Combined weight of rubber, after dialysis, from Exps. 2 and 4 = 115 mgm.

To establish that the radioactivity observed was due to high molecular weight polyisoprene and not to impurities in the rubber as radioassayed, the contents of two counting flasks (Exps. 2 and 4) were evaporated to dryness as a thin film and extracted with boiling ethanol for 16 hr. The rubber was then dissolved in chloroform containing 3 per cent w/w of ethanol; the solution was centrifuged to remove traces of insoluble material and dialysed against three changes of chloroform–ethanol solution for 96 hr. using alkali-treated cellulose membranes[5] which were readily permeable to β-carotene. The rubber was then ozonized by a high-efficiency procedure developed by Barnard and McSweeney (unpublished work) and converted to lævulinic 2 : 4-dinitrophenylhydrazone which was purified via the sodium salt, recrystallized twice from hot acetic acid and radioassayed by a standard gas counting technique[6] with a counter having an efficiency of 43 per cent.

The yield of lævulinic acid dinitrophenylhydrazone after reprecipitation from the sodium salt was 80 per cent, based on the weight of dialysed rubber taken for ozonolysis, and the observed specific activity of the twice recrystallized lævulinic acid dinitrophenylhydrazone was 736 c.p.m./m.mole carbon dioxide. This is equivalent to a total count-rate of 31,700 c.p.m. in the combined sample of dialysed rubber from Exps. 2 and 4, or 97 per cent of the total radioactivity initially added to the latex. Only the formation of a 1 : 4-poly*iso*prene could give the observed retention of activity, and if degraded to lævulinic acid with the same high efficiency as the inactive rubber, would need to have a molecular weight of at least 2,000. The dialysis procedure used during purification indicates that the synthesized material is in fact of considerably higher molecular weight. The results also show that the conversion of *iso*pentenyl pyrophosphate into products other than the high molecular weight polyisoprene must have been negligibly small.

We wish to thank Dr. Konrad Bloch for the generous gift of a sample of [1-^{14}C]-*iso*pentenyl pyrophosphate.

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Nature and Composition of Dental Plaque

ALTHOUGH dental plaque is often mentioned in dental literature, its chemical composition has received little attention. Current literature frequently refers to "dentobacterial plaque" with the implication that it consists principally of bacteria. Manly[1], however, brushed teeth with a detergent prior to the collection of a film, which he called "brown pellicle". This material was free from micro-organisms and was considered to be principally protein. Vallotton[2] studied a similar deposit and found 8·9 per cent nitrogen and 24·8 per cent ash. Earlier, Dobbs[3] had also detected carbohydrate and lipid materials, in addition to protein and inorganic substances.

We have collected plaque from dentures after thorough brushing in a stream of running water. This material shows characteristics similar to those of the deposits described by Manly[1] and Vallotton[2]. Samples examined microscopically and by culture in various media were virtually sterile. Specimens sectioned in a vertical plane and examined by standard histochemical techniques appeared quite structureless except for occasional cocci on the surface. If, however, the washing stage was omitted, moderate growth was observed in the cultures. It seems likely, therefore, that bacteria, rather than constituting the main bulk of the plaque, are located at its surface. Different conditions may, of course, pertain on natural teeth.

Plaque collected after washing as described was dried *in vacuo* and stored at 2° C. until required. Powdered, weighed samples were stirred in aqueous suspension at room temperature using as a stirrer a small inverted filter funnel packed with 'Dowex 50'; the lower end being closed by a dialysis membrane. After 5 hr. the suspension was filtered and the residue dried and weighed. As a check that this method successfully removed all the free amino-acids, plaque was treated with dinitrofluorobenzene and thoroughly washed with organic solvents and water. Before the ion-exchange treatment dinitrophenyl derivatives of most of the common amino-acids were observed in the washings, but afterwards none was detected. In order to estimate inorganic material and free amino-acids it was necessary to ash samples before and after the ion-exchange treatment since this latter also removed most of the inorganic constituents. Lipid material was then estimated by extraction with chloroform/methanol (2 : 1) at room temperature. The resulting grey-brown powder contained 14 per cent nitrogen and only 2·0 per cent inorganic ash. Since all the common protein-forming amino-acids were liberated on hydrolysis this is afterwards referred to as 'plaque-protein'.

Although only occasional traces of carbohydrate were detected after hydrolysis of plaque-protein, extensive humin formation was usually observed. Hydrolysis in the presence of 'Dowex 50'[3] was found to be partially effective in preventing this. By adding known amounts of glucose to plaque-protein samples prior to hydrolysis, it was possible to estimate the extent of destruction of this carbohydrate. After hydrolysis the aqueous eluate from the resin was examined for hexoses and pentoses by the anthrone reaction[5,6] and by paper chromatography according to Wilson's method[7]. The acidic eluate was examined by the Elson–Morgan reaction both in solution and on paper chromatograms. Analytical results are summarized in Table 1.

Table 1. COMPOSITION OF DENTAL PLAQUE

Constituent	Mgm. percentage of dry plaque
Inorganic Ca^{2+}	2·7
Mg^{2+}	0·57
PO_4^{3-}	3·8
Total ash	10·6
Nitrogen (micro-Kjeldahl)	
Total	12·6
Protein nitrogen	11·1
Free amino-acids	11·6
Lipid	1·9
Carbohydrate	
Glucose	3·5–5
Pentoses	*c.* 1·0
Hexosamines	< 0·1

Aspartic acid and arginine were shown to be the principal N-terminal amino-acid residues by the dinitrofluorobenzene procedure[8]. These are present in approximately equimolecular amounts and only traces (< 10 per cent of the aspartic acid) of other N-terminal residues (serine and alanine) were observed. Treatment of a dinitrophenyl-plaque-protein suspension with chymotrypsin substantially reduced the amount of dinitrophenyl-arginine found after subsequent acid hydrolysis. By means of a talc column[9] it was possible to isolate from the soluble portion of the digest a dinitrophenyl-peptide, which yielded dinitrophenyl-arginine on hydrolysis. The protein mixtures obtained from saliva by centrifugation or precipitation showed, as expected, a wide range of different N-terminal amino-acid residues. These results suggest quite a marked degree of homogeneity in plaque-protein and imply a certain degree of selectivity in the mechanism involved in plaque deposition.

We are indebted to Mr. B. Steiger for the bacteriological and histochemical work and to Miss J. C. Riggott for technical assistance.

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A New Leaf Copper Protein 'Plastocyanin', a Natural Hill Oxidant

THE occurrence in *Chlorella ellipsoidea* of a new copper protein, able to undergo reversible oxidation-reduction reaction, has been reported by one of us (S. K.)[1]. The methods of extraction and purification as well as some properties characterizing the substance have been described. This substance is distinguished from any of the oxidases by the fact that in its reduced form it is unable to react directly with molecular oxygen. Similar substances have since been found to occur in the green leaves of various higher plants (for example, spinach and *Chrysanthemum coronarium*). In view of its localization in the chloroplasts, and its characteristic blue colour in the oxidized form, the name 'plastocyanin' is proposed for this copper protein. Further work has been done to elucidate the behaviour of plastocyanin during photosynthesis. In this communication, the photoreduction (Hill reaction) of this substance and its effect on the photo-reduction of other known Hill reagents are briefly described.

Spinach, obtained from a local market, served as the starting material for the preparation of plastocyanin and grana. Details of the procedures devised for extraction and purification of plastocyanin will be published elsewhere. In brief, the substance was extracted from the acetone powder of ground spinach leaves with 0·01 *M* phosphate buffer, *p*H 7·0, and the extract was fractionated with ammonium sulphate. The precipitate obtained between 50 and 100 per cent saturation with ammonium sulphate was dissolved and dialysed against 0·02 *M* phosphate buffer, *p*H 7·0, and placed on a column of diethylaminoethyl cellulose equilibrated with 0·02 *M* phosphate buffer, *p*H 7·0. The column was successively washed with increasing concentrations of phosphate buffer, *p*H 7·0, and finally the substance was eluted with a 0·2 *M* solution of the same buffer. By repeating the ammonium sulphate fractionation and column chromatography, a highly purified preparation of plastocyanin is obtained. The purity index of the preparation thus obtained, expressed as the ratio of the extinction at 280 mμ to that at 597 mμ (maximum of absorption in the visible region), was found to be as low as 1·2.

To prepare grana, 100 gm. of spinach leaves were homogenized for 40 sec. in a Waring blendor with 150 ml. of sucrose solution (0·4 *M* sucrose, 0·01 *M* potassium chloride and 0·01 *M* phosphate, *p*H 7·0). The homogenate was forced through cloth and the resulting fluid fractionated by centrifugation, and the pellet obtained between 1,000*g* (5 min.) and 10,000*g* (10 min.) was washed three times and finally re-suspended in the sucrose solution.

To test the photo-reduction of plastocyanin, the reaction mixture containing plastocyanin and washed grana was illuminated with a tungsten lamp (100 W.) at a distance of 15 cm. The reduction of plastocyanin was followed by measuring the optical density at 597 mμ alternately under light and dark conditions (Fig. 1). In the light, plastocyanine was steadily reduced until complete reduction was achieved. During the dark periods between the light periods there was no reduction, nor was any oxidation of the substance observed. On addition of ferricyanide after the completion of photo-reduction, the recovery of optical density at 597 mμ to the original level was readily obtained, thus indicating that the observed optical changes were due to the reduction of plastocyanin. Potassium cyanide, at a concentration of 10^{-3} *M*, was found to be without effect on this photo-reduction. The reaction was also scarcely affected by sodium azide at the same concentration. On the other hand, *o*-phenanthroline, known to be a potent inhibitor of the Hill reaction, showed a marked inhibition, suppressing the reduction-rate by 75 per cent at 10^{-3} *M*. Although a direct proof of evolution of oxygen accompanying photo-reduction of plastocyanin has not yet been obtained, it may be justifiable to consider the substance under investigation to be a natural Hill oxidant occurring in the chloroplasts of green plants.

In another set of experiments the effect of plastocyanin was observed on the rate of the Hill reaction when other Hill reagents served as oxidants. Although the time-course of the reaction was somewhat complicated by an initial lag-phase, about two-fold stimulation in the rate of reduction of the dye was achieved by the addition of relatively minute amounts of plastocyanin in addition to the main Hill reagent (2,6-dichlorophenol indophenol, in this case). The experimental results shown in Fig. 2 may be best accounted for by assuming that plastocyanine in the initially oxidized state exerts a competitive inhibition of the reduction of the indophenol dye, while in its increasingly reduced state it entails an acceleration of reduction of the dye, presumably by acting as an electron carrier. In fact, it will be seen from the two lower curves in Fig. 2 that the duration of the lag period is roughly proportional to the amount of added plastocyanin. In similar experiments, but with mammalian cytochrome *c* as Hill oxidant, the addition of plastocyanin also caused a strong stimulation of photo-reduction.

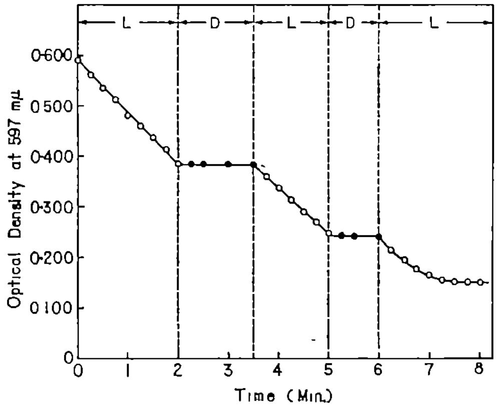

Fig. 1. Photo-reduction of plastocyanin in the presence of spinach grana. Reaction mixture: 150 μmoles phosphate, *p*H 7 0; 30 μmoles potassium chloride; 1,200 μmoles sucrose; plastocyanin (optical density at 597 mμ, 0 44) and grana equivalent to 9 μgm. chlorophyll; total volume 3·0 ml. Reduction of plastocyanin was determined spectrophotometrically at 597 mμ in air (15° C.). Reaction mixtures were illuminated with a 100-W. tungsten lamp (*L*) or kept dark (*D*) for the periods indicated, and then readings of optical density were made by inserting the cuvette into the cell-holder of the spectrophotometer

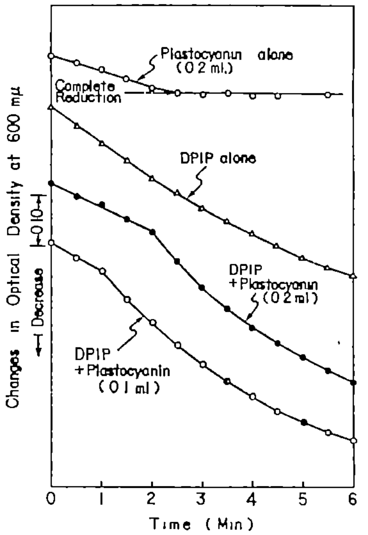

Fig. 2. Effect of plastocyanin on photo-reduction of 2,6-dichlorophenol indophenol (*DPIP*) in the presence of spinach grana. Reaction mixture: 150 μmoles phosphate, *p*H 7 0; 30 μmoles potassium chloride; 1,200 μmoles sucrose; 0 7 μmole 2,6-dichlorophenol indophenol; grana equivalent to 4 9 μgm. chlorophyll, and indicated amounts of plastocyanin (0 1 ml. solution containing plastocyanin equivalent to optical density of 0 034 at 597 mμ; light path 1 cm.); total volume 3 0 ml. Reactions were measured as in Fig. 1; optical density determined at 600 mμ. Curves were plotted on optical density scale at arbitrary locations on the ordinate

These experimental results suggest that the new copper protein under investigation may play an important part in the photosynthetic mechanism in green plants.

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PHYSIOLOGY

Alteration of the Growth of Mammalian Cells *in vitro* by Ecdysone Extract

In insects metamorphosis is controlled by a balance between juvenile hormone (neotenin) and metamorphosis hormone (ecdysone) with trophic neurosecretion responsible for periodicity[1-4]. Work on the reason for regression of atypical growths in *Drosophila*[5] during the larval stage implicated this hormonal mechanism in the observed behaviour[6,7]. As a result of this work, investigation of the possibility that hormones in invertebrates may have an effect on mammalian cells is in progress. In earlier work[8], there were suggestive regressions of transplanted tumours in mice when ecdysone was injected into individuals bearing transplants. Although the large amount of hormone required render this type of testing impractical except on a limited scale, the results were sufficiently encouraging to stimulate additional studies *in vitro*.

Ecdysone was extracted from fresh chrysalides of *Bombyx mori* preserved in methanol, and the fractions were tested for activity by utilizing the *Calliphora* test[9] (Parke, Davis and Co. kindly supplied facilities and personnel for initial phases of the extractions). Cultures of both sarcoma 180 and embryonic, murine fibroblasts were tested for possible alteration in growth after treatment with graduated concentrations of ecdysone, which was titrated in balanced salt solution after passing through a Seitz filter. Sarcoma 180 was grown subcutaneously in mice, and the tumours were removed aseptically on the fifth to seventh day following explantation when a diameter of 0·6–0·8 cm. was reached. After the tissue was minced in warm, balanced, salt solution containing penicillin and streptomycin, fragments 1·0 mm. in diameter were used for culture. The fragments were grown by the double coverslip method in chick-plasma clot, using medium consisting of Eagle's basal medium, calf serum, and chick-embryo extract. Similar methods were used to grow embryonic fibroblasts. The behaviour of experimental and control cultures was followed daily by means of photomicrographs, stained preparations, and time-lapse, phase cinémicrographs. Dosages of crude ecdysone used were 1, 12, 25 and 62 *Calliphora* units.

The growth of cultures of sarcoma 180 was obviously suppressed within 24 hr. in doses of 12 units or more, the effect increasing with concentration. An interesting aspect of this work has been rapid, initial growth of cells treated with ecdysone, with subsequent inhibition. Evidence of this is more subjective, whereas the evidence of inhibition with the extract is incontrovertible. Three criteria were used in evaluating the effect of the hormone. They were: the growth curve of the cells, the number of cells and the time the cells die. Treated cells reached a plateau of growth much earlier than the control cultures, which continued to grow. Also the number of cells was greater in control cultures, and, finally, death of the cells occurred earlier in the series treated with ecdysone. Results from experiments with the lowest dose found to be effective demonstrate the method of scoring (Table 1). The results obtained in 56 experiments with sarcoma 180 are included in Table 2.

Certain morphological and dynamic characteristics of the cells changed with the addition of ecdysone. There was diminution in size as well as in the number of cells, and the amount of cytoplasm as well as the number of pseudopodia were reduced in those treated.

Table 1. INHIBITION OF SARCOMA-180 WITH 12 *Calliphora* UNITS OF ECDYSONE

Exp.	Day 1	Day 2	Day 3	Day 4	Day 5
*C*1	+	+++	++++	++++†	++++*
*C*2	+	++	++++	+++++†	+++++*
*C*3	+++	++++	++++	++++†	++++*
*C*4	+++	++++	++++	+++++†	+++++*
*E*1	++	++†	++	++*	++
*E*2	+	+†	++	++*	++
*E*3	+++	+++†	+++	+++*	+++
*E*4	+++	+++†	+++*	+++	+++
*E*5	+++	+++†	+++	+++*	+++

C, Control; *E*, experimental. †, Cessation of growth. * Death. +, growth of few cells attached to explant; ++, growth of cells near explant; +++, growth of many cells some distal to explant; ++++, growth of many distant cells, intracellular bridges; +++++, formation of sheets of cells.

Table 2. EFFECT OF ECDYSONE ON SARCOMA-180 *in vitro*

No. of exp	Concentration ecdysone		Summary of results
	µgm. per culture	*Calliphora* units	
5	2·6	1	No alteration of growth
29	26·0	12	Inhibition begins day 2*
17	50·0	25	Inhibition begins day 1*
5	130·0	62	Almost complete suppression*

* Initial enhancement.

The distance which the cells migrated from the fragment was also diminished after treatment, and mobility of the cells was reduced by ecdysone as part of the general inhibition. Nuclear pycnosis, rounding up, and early death of cells were augmented with treatment, and an interesting granularity in late-treated cultures was very striking.

Ecdysone also altered the growth pattern of embryonic fibroblasts. The onset of growth was delayed by increasing concentration of the hormone until total inhibition occurred. The results of 63 experiments are summarized in Table 3. No initial stimulation of growth of these cells was observed.

Table 3. EFFECT OF ECDYSONE ON EMBRYONIC FIBROBLASTS *in vitro*

No. of exp.	Concentration ecdysone		Summary of results
	µgm. per culture	*Calliphora* units	
12	12	6	Growth begins day 2
24	25	12	Growth begins day 3
12	45	22	Growth begins day 4
15	80	40	Total inhibition

The presence of a threshold and the increase in effect up to total inhibition with increased dose of hormone all indicate that crude ecdysone inhibits the growth of both sarcoma 180 and embryonic fibroblasts. Two differences in the type of inhibition of sarcoma 180 in comparison with embryonic fibroblasts are of interest. First, there is a suggestion of initial enhancement of growth, with subsequent inhibition, in the case of sarcoma 180 which was not observed in the growth pattern of treated, embryonic fibroblasts. Secondly, sarcoma 180 grows initially in the culture, and the length of this period of growth is curtailed increasingly as the concentration of hormone is augmented. On the other hand, the time at which growth of embryonic fibroblasts begins is extended by the hormonal preparation from a threshold to complete inhibition.

Whether the increased granularity in older, treated cultures has some significance in connexion with the effect of the hormone on alteration of intracellular, enzymic activity is under scrutiny. Since a crystalline preparation was not used, the exact ingredient of the preparation responsible for the effect cannot be identified with certainty. However, the impression that the effect paralleled the concentration of the hormone according to bioassay rather than the weight of the preparation suggests that the hormone itself is responsible. One of the more interesting questions raised by this work is whether this mnemonic phenomenon with phylogenetic implications will become a useful means of altering certain aspects of cellular physiology at will. The possibility of introducing biological regulators into systems where they are effective, yet not normally present or active, is a provocative development.

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Serum Iron and its Transport Mechanism in the Fowl

LITTLE work seems to have been done on iron metabolism in the fowl, especially in the field of transport by serum. First, we investigated this mechanism in various mammals[1] and later in the fowl[2-4]. Iron in the blood is attached to β_1-globulin (transferrin or siderophilin), the transport mechanism of which seems to be common to higher vertebrates; but we found in the hen bird that there is a special behaviour during the laying season[3,4].

It has been shown[5] that during the laying season the hens had a high level of serum iron. Using Ramsay's techniques[6,7], we were able to show that the values of serum iron are even higher than the total iron-binding capacity in the serum of laying hens and laying ducks[4].

We have studied four species: *Gallus domesticus*, *Meleagris gallopavo*, *Anas platyrhynca*, var. Kaki-Campbell and *Anser anser*. In each we determined serum iron values and the total iron-binding capacity using Ramsay's methods[6,7]. In addition, we have also calculated the saturation coefficient for siderophilin.

Table 1. SERUM IRON, TOTAL IRON-BINDING CAPACITY AND SATURATION COEFFICIENT IN CERTAIN BIRDS

Species	Sex and No. of specimens	Serum iron (γ Fe per cent)	Total iron-binding capacity (γ Fe per cent)	Saturation coefficient (per cent)
Meleagris gallopavo	♂ 10	70±16·2 (σ=48·7)	302·1±12·2 (σ=36·6)	24·7±5·4 (σ=16·2)
	♀ NL 11	104·9±14·5 (σ=45·2)	315·2±14·8 (σ=46)	33·6±4·4 (σ=13·9)
Gallus domesticus	♂ 23	102·5±4·8 (σ=22·4)	165±14·2 (σ=65·5)	67·8±3·9 (σ=18·1)
	♀ NL 23	129±10·4 (σ=47·8)	262±15 (σ=69)	51·7±4·1 (σ=18·9)
	♀ L 16	500±42·4 (σ=165·5)	272±21·8 (σ=85·1)	198±21·5 (σ=83·9)
Anas platyrhynca	♂ 6	159·1±50 (σ=110·2)	634·5±54·5 (σ=120·1)	25·5±7·3 (σ=16·1)
	♀ NL 6	132±18·5 (σ=40·5)	472±54·2 (σ=108·5)	28·4±2·1 (σ=4·3)
	♀ L 19	1,065±68 (σ=286·6)	504±32·9 (σ=131·8)	227·5±18·1 (σ=72·7)
Anser anser	♂ 7	163·8±16·1 (σ=38·7)	547·3±25·1 (σ=60·3)	29·9±2·3 (σ=5·5)
	♀ NL 6	160±10·5 (σ=23·1)	561±36·5 (σ=80·3)	28·9±1·9 (σ=4·2)
	♀ L 1	1·260	705	178·7

NL = not laying. L = laying.

Laying females show a considerable rise in serum iron, giving values higher than those for males and for non-laying females; these values are also different from those which were found in different mammals[1]. This rise has been observed especially in ducks and geese (Table 1). The difference in total iron-binding capacity is clearly marked in these species, although it is not so evident as the corresponding serum iron values. However, the differences for females during and outside the laying season for the same species are not statistically significant.

The values for the saturation coefficient of siderophilin are much increased (100 per cent) in the laying female.

It is surprising to see how the serum iron values are approximately doubled compared with the corresponding total iron-binding capacity. This resulted from the application of Ramsay's method[7] in the determination of total iron-binding capacity, where the use of magnesium carbonate to retain surplus iron causes at the same time a partial elimination of circulating iron.

Table 2. SERUM IRON BEFORE AND AFTER TREATMENT WITH MAGNESIUM CARBONATE AND TOTAL IRON-BINDING CAPACITY IN LAYING BIRDS

Species	No.	Serum iron (γ Fe per cent)	Serum iron after treatment (γ Fe per cent)	Total iron-binding capacity (γ Fe per cent)
Gallus domesticus	7	570±68·8 (σ=165·2)	244±36·6 (σ=88)	226·4±41·7 (σ=100·1)
Anas platyrhynca	11	1,183·1±136·7 (σ=424)	578·7±80·4 (σ=249·5)	533·2±59·6 (σ=185·3)

The determination of serum iron in serum previously treated with carbonate (200 mgm. magnesium carbonate + 2 c.c. distilled water + 1 c.c. serum, during 30 min. and centrifugation) gives a value for the laying female of approximately 50 per cent of that for untreated serum (Table 2). On the other

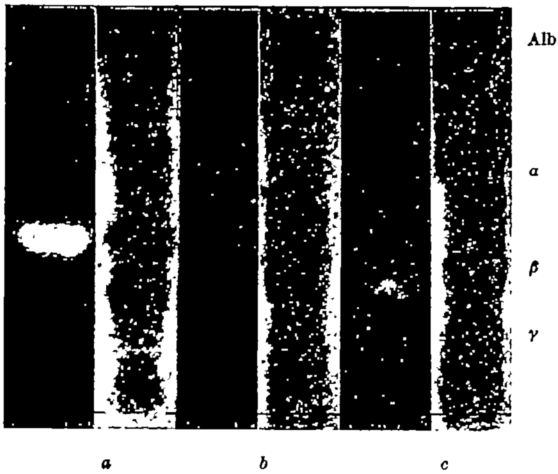

Fig. 1. Electrophoretic bands and their corresponding autoradiographs of the following sera: (*a*) Serum of a laying duck to which has been added iron-59 *in vitro* (1 ml. serum + 2 μgm. (12 μc.)). (*b*) Serum of a laying hen which has previously received by injection 2 μgm. iron-59 (12 μc.). (*c*) Serum of a laying hen to which has been added iron-59 *in vitro* (2 ml. serum + 2 μgm. (12 μc.))

hand, there are no significant differences using serum from females not in season or for serum from males treated in the same way.

It is to be noticed that the average value of total iron-binding capacity does not differ statistically from the serum iron concentrations determined after treatment with magnesium carbonate (Table 2).

In the laying fowl, iron metabolism shows a special character and its transport through the blood presents special characteristics. Two possibilities may be considered : (*a*) that iron travels bound exclusively to siderophilin, with one fraction firmly bound (total iron-binding capacity), and the other fraction more weakly bound and easily separated by the action of carbonate ; and (*b*), that besides the siderophilin other protein fractions take part in the transport of iron.

Recent work with iron-59 indicates that both mechanisms are in operation. In laying ducks, the addition of iron-59 *in vitro* to the serum shows constant fixation to the β-globulin (Fig. 1*a*), and the same behaviour is observed on injecting the radioactive iron into the laying hen (Fig. 1*b*). On the other hand, the addition of iron-59 *in vitro* to serum of laying hens is fixed to all the globulins, but preferentially to the β-fraction (Fig. 1*c*).

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Evidence for the Granular Localization of Posterior Pituitary Hormones

ENDOCRINE organs are able to maintain high concentration gradients between their secretory cells and the extracellular fluid. The mechanisms for maintaining this gradient are not clear, although restraining the hormones within a semipermeable membranous structure could sub-serve this function. Herlant[1] and Brown and Hess[2] have shown that many hormones of the anterior pituitary are stored in a particulate form. In addition, the work of Hillarp *et al.*[3] and Blaschko and Welch[4] indicates that adrenaline and noradrenaline are stored in granules within the adrenomedullary cells.

There is abundant histological and morphological evidence that the neurohypophyseal hormones are found in association with material of a granular or colloidal nature. Rosenfeld[5] found that following long-term ultracentrifugation oxytocic and pressor activity was confined predominantly to the sediment.

The following experiments indicate that by a relatively simple centrifugation technique a sedimented fraction can be obtained from sucrose homogenates of neurohypophyses that contains very much more of the antidiuretic hormone and oxytocic hormone than does the supernatant. When distilled water is added to this sediment it releases the hormones. This suggests that they are stored in the neurohypophyses within a membranous structure.

Cattle neurohypophyses were removed within 60 min. of killing. The glands were kept chilled while being pressed through a 0·5-mm. porcelain sieve. The pressed tissue was then homogenized in 0·55 *mM* sucrose using a power-driven plastic rod within a loose-fitting glass test-tube. Comparative experiments suggested that this concentration of sucrose was more suitable than 0·25 *M* or 0·88 *M* sucrose or saline. The homogenates were then centrifuged at 1,000*g* for 10 min. The sediment was discarded and the supernatant recentrifuged at 50,000*g* for 20 min. The sediment was washed five times with sucrose, and after the last wash the sediment was resuspended in distilled water of the same volume as the last sucrose wash. The sediment was then removed by centrifugation at 50,000*g* and the hormonal activities determined in the supernatant from the last sucrose wash and in the distilled water. The antidiuretic activity was determined by Dr. Bengt Andersson[6] on the hydrated goat. The oxytocin assays were performed on the rat uterus. The chicken blood-pressure method was also used. The results are expressed as total activity in the water-ruptured granules (sediment) or in the supernatant from the sucrose-washed granules (supernatant).

Table 1. UNITS OF HORMONAL ACTIVITY OBTAINED FROM 2·5-GM. CATTLE NEUROHYPOPHYSEAL TISSUE

	Sediment *B.P.*-6	Supernatant *B.P.*-5
Antidiuretic activity	120	3·7
Oxytocic activity	108	0 60

The results indicate that in the gravitational fields employed the hormonal activities were predominantly located in the sedimentable fractions. The release of the humoral activity from the sediment by distilled water suggests an osmotic rupturing of a membranous structure, as has been shown for the catechol-containing granules.

It is interesting to speculate on the possible physiological significance of the granular localization of hormones. As a working hypothesis, we would suggest that the adequate stimulus to an endocrine gland may selectively alter the integrity of the granular membrane restraining this gland's hormonal secretions.

We wish to thank Dr. Bengt Andersson for assaying the antidiuretic activity in our preparations. The senior author is grateful to Prof. U. S. von Euler for the opportunity of carrying out this work in his laboratory.

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Mucopolysaccharides and Thyroid Function

In diseases with intense tissue disintegration, in agreement with reports in the literature[1,2], the mucopolysaccharide (hexosamine)-level of blood was found to increase : thus in tuberculosis and in cancer it was found to increase from the normal level of 83 mgm. to 120–160 mgm. per 100 ml. In tuberculosis[3] and C_3H mouse carcinoma[4], the local formation of neutral mucopolysaccharides could be demonstrated. The neutral mucopolysaccharides—formed in the hypoxybiotic areas of the diseased tissues—are drained through the lymphatic vessels. They can be traced in the blood, and so the increase of the mucopolysaccharide content can be interpreted in terms of a pathological mechanism. It is known that, after healing, the mucopolysaccharide content returns to the normal level.

The question arose as to which organ or neuroendocrine system regulates the normal mucopolysaccharide content of the serum. According to results in the literature, the mucopolysaccharide-level is increased in myxœdema[5,6]. Attention was therefore focused on the thyroid gland. The colloid of the thyroid gland gave a positive reaction with the periodic–Schiff reagent. Thus the possible role of the thyroid gland became more comprehensible. The effect of some hormones has already been discussed by Boas and Foley[6] and by Dorfman and Schiller[7].

We investigated the mucopolysaccharide content of 16 hyperthyroidic patients of the I. Medical Clinic of Debrecen. We used the method of Elson and Morgan described by Winzler and modified by Szabolcs and Tankó[8]. By the latter modification—nearly the whole procedure is carried out in the same tube—the limit of error is less than 3 per cent. The results for these patients are represented in Fig. 1. Some of the patients were only slightly hyperthyroidic, or had nearly normal thyroid function. However, the average mucopolysaccharide content was considerably less than the normal level. On the other hand, the mucopolysaccharide content of the blood of hypothyroidic patients was found to be higher than the normal, and considerably higher than the mucopolysaccharide-level of hyperthyroidic patients.

In evaluating the significance of the mucopolysaccharide-level of a patient suspected of hypothyroidism, we have to consider that a high mucopolysaccharide-level occurs not only in cases of myxœdema but also in several diseases with increased disintegration of tissues (tuberculosis, carcinoma, rheumatism, pneumonia, etc.). Thus a high mucopolysaccharide-level (caused, for example, by pneumonia) may eventually mask hyperthyroidism. However, a low mucopolysaccharide content is found to be connected with hyperthyroidism, provided that there is no insufficiency of liver or kidney. To make a diagnosis, several other diagnostic methods should be tested.

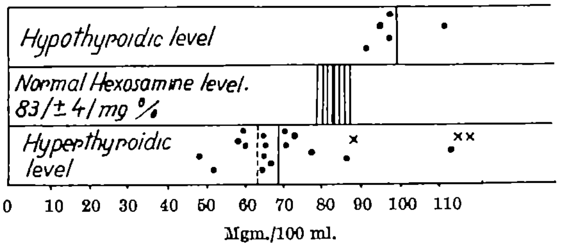

Fig. 1. Diagram illustrating the hexosamine-level of hypothyroidic (average 99·6 mgm. per 100 ml.) and hyperthyroidic (average 68·9, with correction 65 mgm. per 100 ml.) patients. + and + + , two weeks later the hexosamine-level of these patients proved to be 70 and 68 mgm. per 100 ml., respectively

We wish to direct attention to the connexion existing between the local or general hypoxybiosis, the formation of mucopolysaccharides and thyroid function.

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Experimental Data concerning the Connexion between Thyroid Function and Mucopolysaccharide (Hexosamine)-Level

In the preceding communication it was demonstrated that the mucopolysaccharide content of the blood (normally 83 ± 4 mgm. hexosamine per cent) is decreased in hyperthyreosis (65 mgm. per cent on average) and increased in myxœdema (100 mgm. per cent on average). We have tested these observations on experimental animals.

Experiments on rats applying heat and cold stress failed to give evaluable results. Thyroidectomy was carried out on 6 dogs. Their mucopolysaccharide (hexosamine)-level was found to increase within 3–6 weeks by 44 per cent, and was accompanied by clinical signs of myxœdema. Afterwards, thyroid hormone ('Thyroxyne', Roche, 1 mgm. per day, the dogs weighing 10–12 kgm.) was administered for 3–4 weeks ; the increased hexosamine-level of the dogs, which fluctuated for one or two weeks, decreased below the original normal level. Methylthiouracyl (0·05 gm. 4-methyl-2-thiouracyl per kgm.) was administered to a further 6 dogs (10–12 kgm.). The hexosamine-level of these dogs increased within 2–5 weeks by an average of 30 per cent. Thereafter these dogs received also thyroxine (1 mgm. per day continuously). Three of the dogs died prematurely. The hexosamine-level in the remaining three dogs decreased within 2–8 weeks to an average of 72 per cent of the original level. Administration of thyroxine to 4 untreated dogs produced a decrease in the hexosamine-level. The statistical evaluation of these two experiments gave a significant difference (Prof. Gyires, Department of Applied Mathematics). The results obtained on untreated dogs were not evaluated statistically, the number of the experimental animals being too small, and the changes in these experiments were only in one direction.

These experiments showed that experimental thyroidectomy (6 dogs) and methylthiouracyl treatment (6 dogs) caused a significant increase of the mucopolysaccharide (hexosamine)-level of the blood serum, whereas thyroxine treatment (11 dogs) brought about a significant decrease of the mucopolysaccharide-level of the serum. It may be added that thyroxine still had this effect even after thyroidectomy.

Our previous observations on man on the correlation of the decrease of the hexosamine-level in cases of hyperthyreosis were confirmed by our experiments on dogs. By the administration of thyroxine, the hexosamine-level could be decreased. On elevating the hexosamine-level by previous thyroidectomy or by prolonged administration of methyl thiouracyl, the effect of thyroxine became more manifest. By using the method of thyroidectomy, we were able to confirm the findings of Boas and Foley that thyroidectomy on rats produced a sustained increase in the hexosamine-level of the blood. Our experiments on dogs using thiouracyl and our observations on man regarding myxœdema were supported by the experimental results on hyperthyreosis.

Thus we conclude, as regards dogs and man, that the decrease of thyroid function results in an elevation of the mucopolysaccharide (hexosamine)-level, while the sustained increase of thyroid function results in a decrease of the mucopolysaccharide (hexosamine)-level of the blood.

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Identification of a Compound found during Separation of Purines in Human Urine

The purine bases of normal human urine have been separated, identified and quantitatively estimated[1-3]. During these investigations several additional unknown compounds appeared with the purines; they were separated and designated *E*, *W*, *S* and *Z*.

In our search for abnormal purines in the urine of patients with cancer and leukæmia, compound *Z* was found in high concentration in the urine of one leukæmia patient. From examination of a limited number of urine samples, it appeared that normal subjects and leukæmia patients in remission excreted large amounts of this material when compared with patients with cancer and active leukæmia. Identification of this compound was therefore undertaken.

Compound *Z* was isolated from a urinary concentrate by gradient elution chromatography on a column of 'Dowex 50'[1]. Further purification was accomplished by ascending 2-dimensional paper chromatography. Papergrams containing compound *Z* were sprayed with several reagents, but only the Jaffe reagent gave a positive reaction. The finding of a single red spot with this reagent in the position occupied by spot *Z* (rendered visible by ultra-violet light) suggested that this compound might be creatinine. Overlays of creatinine and *Z* in four different solvents gave no separation of these compounds.

The ultra-violet absorption spectrum of *Z* is unlike that of purines in that it has only end-absorption between *p*H 2 and 5, and above 11. It has, however, an absorption maximum between *p*H 5·8 and 11 at 232 mμ. A pure sample of creatinine exhibits the same ultra-violet absorption characteristics.

The insolubility of a silver–purine complex in acid solution is the basis for Weissmann's[1] separation of purines from creatinine, amino-acids, and pyrimidines. In order to determine whether creatinine could be precipitated with acid silver nitrate, a solution of similar creatinine concentration to that found in the urinary concentrate was prepared; this contained 10 mgm. creatinine/ml. water. Addition of acid silver nitrate gave a precipitate which was filtered, washed, and decomposed with hydrochloric acid under the same conditions as a urinary concentrate. The resulting solution on analysis by the Jaffe reaction contained 0·3 per cent creatinine. From this, it is evident that although 99·7 per cent of the creatinine remains in solution during silver nitrate precipitation, the presence of a high concentration of creatinine in the urinary concentrate is sufficient to exceed the solubility product of the silver–creatinine complex, and therefore the residual 0·3 per cent is carried through the procedure and appears with the purines.

This work was done at the New York Medical College, Flower and Fifth Avenue Hospitals, and was sponsored through the United States National Institutes of Health, Division of Research Grants No. C-2591.

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Muscarinic Receptors

The (+)-isomers of acetyl-α- and acetyl-β-methylcholine are respectively about 10 and 250 times more active as muscarinics than their corresponding enantiomorphs (Table 1). We have recently established the configuration of these (+)-isomers as I and II [1,2]; their configurational identity with (+)-muscarine (III) [3] about C_5 is indicated in Fig. 1.

The muscarinic activity of muscarine is considerably reduced in the isomers in which the CH_2, CH_2NMe_3 or OH groups are inverted[4]. If the quaternary N group and the ether O of (III), lying almost in a plane, form the chief two centres for drug-receptor association (the OH group acting as a secondary association site), then association with receptor must involve the lower surface of (III). Inversion of the methyl group of (III) would then produce a steric barrier against such association. Replacement of this methyl group by a hydrogen atom considerably reduces activity[5], probably because of reduction of

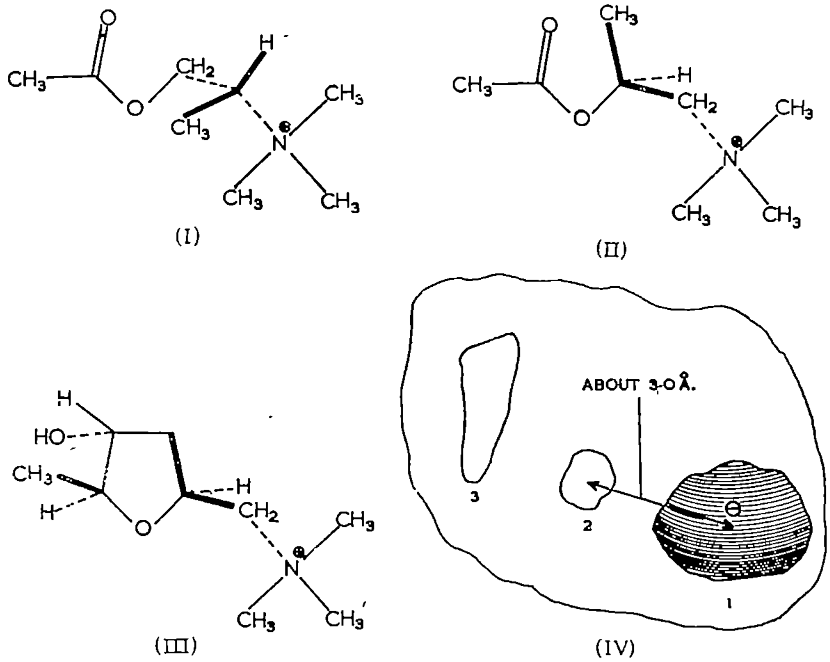
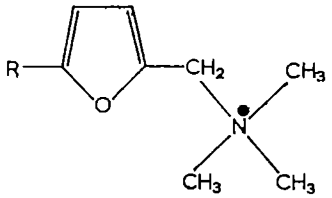


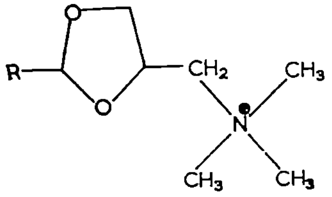
Fig. 1. Configurational relationship of the (+)-isomers of acetyl-α- and acetyl-β-methylcholine with (+)-muscarine. (I) Anionic cavity negatively charged to accommodate quaternary nitrogen. (II) Positively charged point accommodating ether linkage of muscarine or ester linkage of acetylcholine and its analogues. (III) + charged area to accommodate OH of muscarine, $\rangle C{=}O$ of acetylcholine analogues or double bond of furan analogues of muscarine, etc.

electron density on the ether oxygen atom rather than by reduction in the availability of van der Waals' forces for bonding. Similarly, the reduced activity of (V; $R = H$)[6] as compared with (V; $R = Me$) and (VI; $R = H$) with (VI; $R = Me$)[7] is explicable. Because of the rigidity of these molecules, the complementary muscarinic receptor may tentatively be considered as depicted in IV (ref. 8).

The (+)-isomer of acetyl-β-methylcholine can adopt a conformation (I) complementary to that of the depicted receptor (IV), whereas the (−)-isomer cannot do so. The 250-fold greater activity of the former is probably an under-estimate of the true difference in drug-receptor interactions, since acetylcholinesterase is known to hydrolyse the former much more rapidly than the latter[9]. The (+)-isomer of acetyl-α-methylcholine, because of the increase in steric size in the vicinity of the cationic head, unlike acetylcholine, cannot present a surface completely



(V)



(VI)

Table 1. Muscarinic Activities of some Acetylcholine-type Isomers

Drug	Salt	Isomer	Muscarinic activities. Number of mols of drug equivalent to 1 mol. of acetylcholine		Ratio of activity of isomers (+)/(−)	Muscarinic activities. Number of mols of drug equivalent to 1 mol. of acetylcholine	Ratio of activity of isomers (+)/(−)
			Guinea pig ileum Mean	Limits $P = 0{\cdot}05$	Guinea pig ileum	Cat blood pressure	Cat blood pressure
$CH_3COOCH_2\underset{CH_3}{\underset{\vert}{C}}H\overset{+}{N}Me_3$	I′	(±) (−) (+)	49 232 28	35–71 204–264 22–35	8	35 144 13	11
$CH_3COO\underset{CH_3}{\underset{\vert}{C}}HCH_2\overset{+}{N}Me_3$	I′	(±) (−) (+)	1·58 240 1·01	1·35–1·91 208–277 0·94–1·10	240	0·97 202 0·75	270
$CH_3COOCH_2\underset{CH_3}{\underset{\vert}{C}}H\overset{+}{N}Me_2Et$	I′	(±) (−) (+)	264 1,980 170	248–286 1,695–2,310 156–186	12	149 1,920	22

complementary to (IV). The difference in the possible associating surface of the enantiomorphs is much less marked in the α- than in the β-isomers of acetylmethylcholine. Observed differences in the muscarinic activities of the above isomers is therefore explicable in terms of differences in their fit at the muscarinic receptors (IV).

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PATHOLOGY

Neural Tissue and Pulmonary Lesions in Normal and Irradiated Rats Injected with Homogenized Homologous Lung Tissue mixed with Freund's Adjuvant

STARTING from the assumption that auto-immune processes occur in irradiated animals as well as normal animals[1], we have succeeded in inducing experimental allergic encephalomyelitis in irradiated rats[2]. In order to examine the specificity of that phenomenon, several homogenized organ tissues mixed with adjuvant were used, such as brain, liver, lung and pancreas. Of the organs used in these investigations, lung tissue mixed with adjuvant produced the symptoms of experimental allergic encephalomyelitis.

In the experiments 70 albino rats of mixed breed, weighing about 180 gm., were used. The animals received injections of homogenized homologous lung tissue mixed with adjuvant. The ingredients of the antigen mixture were added as recommended by Lipton and Freund[3]. 1·0 ml. of the antigen mixture was divided into four doses of 0·25 ml. each and injected subcutaneously. The doses were given simultaneously in the ventral part of the abdomen, two on each side. The irradiation dose was 600 r. (220 kV., 15 m.amp., 0·5 copper, 1·0 aluminium, r = 40 cm.; the dose-rate was approximately 120 r./min.).

The injections were given 24 hr., 3 hr., 30 min. before, and 30 min., 3 hr. and 24 hr. after, irradiation. One group was not irradiated.

Diagnosis of experimental allergic encephalomyelitis was based on the paralysis of limbs and/or marked atactic gait, arthritis was detected by inspection of paws, and lung lesions were determined by inspection of histological sections stained with hæmatoxylin and eosin. Sections of medulla spinalis were also inspected microscopically, after staining with hæmatoxylin–eosin.

Table 1. INCIDENCE OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) AND ARTHRITIS IN NORMAL AND IRRADIATED RATS BEFORE AND AFTER IRRADIATION

Time of antigen injection	Rats suffering from EAE	Arthritis
Control (not irradiated)	15/20	6/15
− 24 hr.	0/10	—
− 3 hr.	7/10	—
− 30 min.	8/10	2/8
+ 3 hr.	7/10	2/7
+ 24 hr.	7/10	—

The results presented in Table 1 show 70–80 per cent incidence of experimental allergic encephalomyelitis in normal and irradiated rats. Only some of the animals with symptoms of experimental allergic encephalomyelitis had arthritis, whereas pulmonary lesions of various degrees were observed in all animals. The encephalomyelitic symptoms were manifested not earlier than four weeks after injections, which is later than when brain plus adjuvant is used. On the other hand, all the animals recovered in the former instance, whereas this was not the case with encephalomyelitis induced by brain tissue.

Histological sections of brain, medulla spinalis and lung were examined. In brain sections, no alterations could be detected. In the lumbar region of medulla spinalis, typical perivascular infiltrations with cells of mononuclear type, and in some regions diffuse scattered inflammatory cells, were observed. In the pulmonary interstitium and in the interalveolar septa, which became plump and protruded into the alveolar lumen, a strong infiltration of inflammatory cells of mononuclear type occurred.

Although it is not possible to explain the non-appearance of experimental allergic encephalomyelitis in the group of rats injected 24 hr. before irradiation, the incidence of pulmonary lesions and encephalomyelitis in normal and irradiated animals leads us to conclude that auto-immune processes occur also in the irradiated organism.

In this instance it is not possible to explain the antigenic relations between brain and pulmonary tissue. A careful examination of the remaining organs and tissues, and the antigenic relationship between them, will be the subject of further investigations.

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Inhibiting Effect of Nicotinamide and Diphosphopyridine Nucleotide on the Methylcholanthrene Sarcoma in Rats

RECENTLY, Oide[1] reported that when a pellet of nicotinamide was intrasplenically implanted in rats after partial removal of the liver, the numbers of mitoses in the liver were greatly reduced. Further, Fujii and Mizuno[2] demonstrated that this compound has a marked inhibitory effect on the epidermal hyperplasia induced by painting with methylcholanthrene. We attempted to study the influence of the subcutaneous injection of nicotinamide and diphosphopyridine nucleotide (DPN) on the incidence and growth of tumours induced by subcutaneous injection of methylcholanthrene. For this experiment 36 Wistar strain rats weighing about 125 gm. were used. These were divided into three groups, each of which consisted of 12 animals. Rats in each group received subcutaneous injection of 0·25 ml. of cold sterile distilled water (group 1), 1 per cent nicotinamide (supplied by Katayama Chemical Works, Japan) (group 2) or 0·1 per cent DPN (supplied by Nutritional Biochemicals Corp., U.S.A.) (group 3) respectively.

Nicotinamide and DPN were dissolved in cold distilled water. These injections were performed on the back of the rat three times a week until the animals died or were killed. One week after the first injection, all rats were injected subcutaneously in the intrascapular region with 3 mgm. of methylcholanthrene (supplied by L. Light and Co., Ltd., England) in 0·4 ml. of olive oil. The rats of three groups were maintained equally on the usual laboratory diet of whole wheat grains with daily supply of some green vegetables.

During the course of this experiment, all animals showed gradual increase in weight except 3 rats which died of severe pneumonia and lung abscess without any tumour within 100 days after injection of methylcholanthrene. The remaining 33 rats survived more than 100 days after the injection of methylcholanthrene, receiving more than 47 injections of water, nicotinamide or DPN.

Tumour nodules could be first palpated 92 days after injection of methylcholanthrene at the site of injection, and 145 days after injection almost all animals showed tumour nodules. Twenty-three of the tumour-bearing animals died within 167 days; the remaining 10 animals were killed on the 168th day after the injection of methylcholanthrene. The time of '50 and 100 per cent tumour incidence' in each group was as follows: 101st and 120th day in group 1, 122nd and 168th day in group 2, and 124th and over 168th day in group 3. Furthermore, the tumour-incidence rate in groups 2 and 3 was still 30 and 36 per cent respectively, even when that in group 1 reached 100 per cent. From these results, it may be said that the incidence of methylcholanthrene sarcomas was retarded by 3–6 weeks in the groups of nicotinamide or DPN injection, compared with the group of water injection. Average values of tumour weight and tumour index in each group were as follows: 48·1 gm. and 28·1 in group 1, 34·9 gm. and 17·8 in group 2, and 27·6 gm. and 15·7 in group 3. These results denote that nicotinamide and DPN both retarded the growth of tumours. All these tumours were fibrosarcoma, and no difference was observed in histological structure among the groups. That the presence of nicotinamide greatly inhibited cozymase nucleosidase activity in rat brain has been reported by Mann and Quastel[3]. Therefore, it may be probable that nicotinamide injection causes an accumulation of DPN in the cytoplasm, and so exerts its influence by inhibiting the cell division. However, the possibility that nicotinamide enhances the rate of nuclear DPN synthesis and increase of nuclear DPN content results in inhibition of cell division cannot be excluded, since it is expected that nicotinamide serves as a precursor of nicotinamide mononucleotide which is essential for the synthesis of DPN[4]. Thus, the inhibiting effect of nicotinamide and DPN on the incidence and growth of the methylcholanthrene sarcoma observed in the present work may be attributed to such an inhibitory effect on cell division.

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Lipofuscin Pigmentation of Sheep Livers in Australia

ALMOST all sheep, as well as some other animals, grazing in certain districts of Australia, develop a peculiar pigmentation of their livers, portal lymph nodes and to a lesser degree of their lungs, without showing any adverse clinical signs. In the Brisbane abattoir about 25,000 livers (3 per cent) are condemned annually because of this discoloration. The pigmented livers are also seen in the Sydney abattoir, but are completely unknown in Tasmania, South Australia, Victoria and Western Australia, according to information obtained from the chief veterinary officers of these States.

According to observations by veterinary officers, graziers, drovers and other people dealing with animals, the pigmentation is found in all sheep grazing in certain areas of mulga (*Acacia aneura*) country. No such evidence has been encountered outside mulga country, but as stated above, this condition does not occur in Western Australia, South Australia or the Northern Territory, although they contain considerable areas of mulga country.

Local people claim to have noticed the discoloration in the livers of sheep, slaughtered as early as two or three weeks after arrival in this particular type of country, while other sheep, which came with the same group but were slaughtered earlier, had normal 'red' livers. The circumstances in which this pigmentation occurs points very strongly towards a nutritional origin. However, it is not known what constituent of the diet is responsible for the change.

Mulga forms extensive forests, in country between the 8–20 in. annual isohytes. It is one of the most important fodder trees in Queensland, being usually 5–8 m., but sometimes up to 12 m., high. In periods of drought trees or branches are cut down and sheep may be maintained on the leaves for years.

On superficial examination all parts of the affected livers are of uniform colour, which may range from a slight darkening to a deep charcoal grey. Closer inspection of the dark livers reveals a brown to black lace-like network, outlining the liver lobules of peripheral and cut surfaces. Histological examination shows pale yellow to dark brown granules, about

Table 1. SUMMARY OF MOST SIGNIFICANT REACTIONS

	Light liver gran.	Dark liver gran.	Lipo-fusc. heart	Melanin skin eye
1. Dyes				
Neutral red	2–3	2	4	0
Gentian violet	4	4	4	0
Mallory's hæmofuscin	4	3–4	4	0
2. Buffered staining				
Methylene blue *p*H 9·20	4	4	4	2–3
6·94	4	4	4	2–3
2·31	0	0–2	0	3
Methylene blue *p*H after benzoylat. 9·20	3	3–4	3	3
6·94	0	1–3	0	3
2·31	0	0–2	0	4
Methylene blue *p*H after methylation 9·20	1	3–4	0	4
6·94	0	1–4	0	4
2·31	0	0–2	0	4
3. Basic dyes after bleaching				
Nile blue	4	4	4	0
Gentian violet	4	2–4	3	0
Mallory's hæmofuscin	4	1–4	4	0
Giemsa	4	4	4	0
Methylene blue	4	2–4	4	0
4. Fat stains				
Sudan IV	1–2	0	0	0
Sudan black	3	1–2	3	0
Oil red 0	3	0–1	3	0
5. Reduction test				
Bielschowsky silver reaction	0	2–3	0	4
Ferric ferricyan. reduct. test	4	4	4	4
Ferric ferricyanide reduct. test after chromic acid	0	0	0	3
6. Schiff reactions				
Periodic acid–Schiff	4	2–3	4	0–2
Performic acid–Schiff	2	0	2	0
Peracetic acid–Schiff	3	2	3	0

7. Bleaching	Liver pigment	Lipo-fuscin	Melanin epidermis	Melanin eye
After bleaching	27 days, still some pigment visible	16 days, no pigment	3 days, no pigment	12 days, most pigment disappeared, 27 days, no pigment

0, unstained or test negative or inhibited by blocking; 1, almost unstained or test gives a very slight reaction, still regarded as negative; 2, poorly (slightly) stained or test weakly positive; 3, well (distinctly) stained or test well positive; 4, very well (very distinctly) stained or test very strongly positive.

2 microns in diameter, mainly in the liver cells. In the darker livers they are also in the Kupffer cells, in phagocytic cells of the portal tracts and in the lumen of central veins and to a lesser degree in the vessels of the portal tracts.

This pigmentation is usually referred to as 'melanosis', because the pigment has the general microscopic appearance of melanin. However, the condition is different from genuine melanosis, a congenital abnormality, seen occasionally in liver and other organs of slaughtered animals[1]. The microscopic distribution of the pigment is also most unusual for melanin; consequently a full investigation of the nature of this pigment was carried out, and the detailed results will be published in monograph form[2].

Numerous reactions were tried and performed simultaneously on liver tissues and on identically treated control material of skin from a black dog, eyes from sheep and bovine hearts with extensive lipofuscin pigmentation. The reactions shown in Table 1 proved particularly useful for differentiating between lipofuscin and melanin.

(1) *Staining with neutral red, gentian violet and Mallory's hæmofuscin method.* The granules in liver and heart were stained to various degrees, while the melanin in skin and eye was not stained. With Mallory's hæmofuscin method the number of visible granules in the liver was increased due to stained precursors.

(2) *Buffered staining with methylene blue, without and after blocking by benzoylation and methylation.* Only three *p*H values are given in Table 1 although 17 were used, ranging from 9·20 to 2·31.

This reaction separated the pigments distinctly in two groups. Without previous blocking, the granules in liver and heart showed strong affinity to methylene blue in alkaline solution, which lessened with decreasing *p*H. In contrast, the melanin granules stained almost uniformly throughout the *p*H range, and the lowest *p*H even increased their affinity to the dye. Benzoylation reduced noticeably the staining of lipofuscin, but intensified that of melanin. Methylation had the same effect to a much higher degree.

(3) *Staining with basic dyes after bleaching, particularly with Nile blue, gentian violet, basic fuchsin, Giemsa and methylene blue.* In unbleached sections all pigments showed affinity to the above dyes. When the tissues were bleached in a mixture of hydrogen peroxide and alkali, the liver and heart granules reappeared on staining, even when they had been exposed to the bleaching agent for a considerably longer time than necessary for complete bleaching of the pigment. The melanin granules had lost their affinity to the basic dye even before they had been completely bleached.

(4) *Fat stains with Sudan IV, oil red 0, and particularly Sudan black.* The granules in liver and heart were stained with the above dyes in increasing intensity, whereby the paler granules showed a stronger reaction than the darker ones. The melanin granules remained unstained.

(5) *The ferric ferricyanide reduction test after treatment with chromic acid and the Bielschowsky silver reaction.* The ferric ferricyanide reduction test was positive with all pigments. However, when the slides were previously treated with chromic acid it was noticed that the liver and heart granules gave a negative reaction, while melanin was still positive. The Bielschowsky silver reaction differentiated between lipofuscin in the heart, which was not or only slightly darkened, and the melanin granules, which showed distinct blackening. In the liver the light granules remained unaltered, while the dark ones were moderately darkened.

(6) *The periodic, performic and peracetic acid–Schiff reactions.* These reactions were positive to various degrees with the pigment in liver and heart, but negative or almost negative with melanin.

(7) *Bleaching with hydrogen peroxide.* Not only speed of bleaching but particularly the difference between formalin- and alcohol-fixed material was observed. It was noticed, first, that lipofuscin was more resistant to bleaching than melanin. This is a generally recognized technique of distinguishing between lipofuscin and melanin. Secondly, it was observed that lipofuscin bleached more quickly in alcohol than in formalin-fixed tissue, while the reverse was the case with melanin. The pigment in the liver acted like lipofuscin.

As a result of these reactions it has been concluded that the pigment in the livers is lipofuscin at various stages of oxidation, ranging from unstained precursors, which can be made visible by certain reactions, to dark brown and very inert granules, which may be difficult to distinguish from melanin. A number of the techniques used represent an advance in methods of differentiating lipofuscin and melanin.

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RADIOBIOLOGY

Accidental Radioactive Labelling of a Migratory Moth

Radioactive labelling from the Sahara atomic bomb tests (*by* H. B. D. K.). It has been shown in the laboratory that if the larvæ of Lepidoptera are fed on radioactive foliage for a 24-hr. period, the resulting radioactive imagines can be readily identified. Furthermore, for such an isotope as sulphur-35, the count may actually be higher in the imago than it was in the larva. This is due to the increased surface area of the wings[1]. More recently, this method has been put to the test in wild populations[2]. Homometabolous insects, and in particular the Lepidoptera, are in fact ideally suited to this type of labelling.

In February 1960, it was reported that the first of two atomic weapons had been exploded successfully in North Africa. It appeared to me that this might offer an opportunity for the first time of checking whether certain migratory moths, which arrive in Great Britain in the early months of the year, had flown direct from the African Continent. It has not hitherto been possible to show by any experimental devices whether certain species that have their breeding reservoirs in the North African deserts do in fact spread northwards from Africa each year throughout Europe. Accordingly, a notice was placed in many British and Continental entomological journals requesting early migratory specimens of seven species suspected by me of maintaining their winter populations in North Africa but not in Europe.

Of the several hundred specimens received (the majority were the Noctuid moth, *Plusia gamma* L.), one insect only was found to have an increased count under the Geiger-counter. This was a specimen of *Nomophila noctuella* L. (Fig. 1) taken by me in a mercury-vapour trap at Steeple Barton, Oxford, on the morning of March 10. This insect, which was a pale buff colour, unlike the home-bred specimens which occur in the late summer, was one of four taken in Britain about the same date. It consistently gave a count of 15–20 per cent above background. The specimen was therefore subjected to autoradiography for three months, at the end of which time the X-ray films were developed. Because of the increased count, it was anticipated that a larva of this quick-feeding species might have fed on contaminated vegetation and that the resulting imago would show the usual autoradiograph of an individual so treated. The X-ray films, however, showed no such thing, but a single spot of very small size was visible. In view of this, and at the request of the Health Physics Division, Atomic Energy Research Establishment, Harwell, the insect, along with the autoradiographs, was passed over to them, and their findings are reported here. During the earlier investigations, a specimen of *N. noctuella* taken in 1892 was used as a control. It gave negative results throughout.

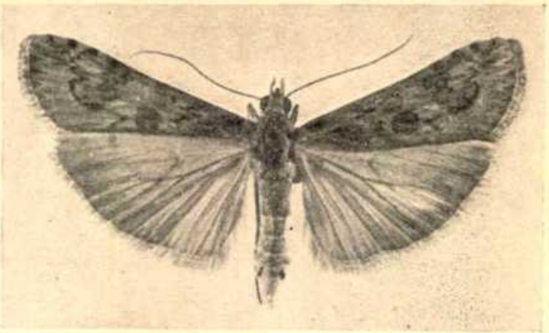

Fig. 1. (× 2½)

Report on the radioactive moth Nomophila noctuella L. (*by* M. J. H.). The appearance of the initial autoradiograph suggested a point source of βγ-activity close to the surface of the thorax of the moth. Subsequent autoradiographs of preparations of this part of the moth resulted in the identification of a single radioactive particle (Fig. 2). This particle is a perfect sphere, 9μ in diameter and of a dark brown colour. It is entirely typical of the particles observed after test explosions on, or near, the surface of the ground and is probably composed of fused silica coloured by traces of metal.

As there had been a long gap in the testing of nuclear devices before February 1960, a particle of this size could not have originated from earlier explosions. Its origin in the Sahara test of February 13 is confirmed by the pattern of its radioactive decay. Several estimates of the disintegration-rate were made using an end-window counter initially, and the size of autoradiograph images later. The decay of mixed fission products is normally expressed in the form $A \propto T^{-n}$, where n is a constant. The scatter of measurements, which involved several different detection systems, is considerable. However, assuming the time of origin to be February 13, the value of n for this particle falls within the range 1·0–1·5. This agrees with observations of the decay of individual fall-out particles. The actual activity of this particle on March 18 was 7 μμc. (beta).

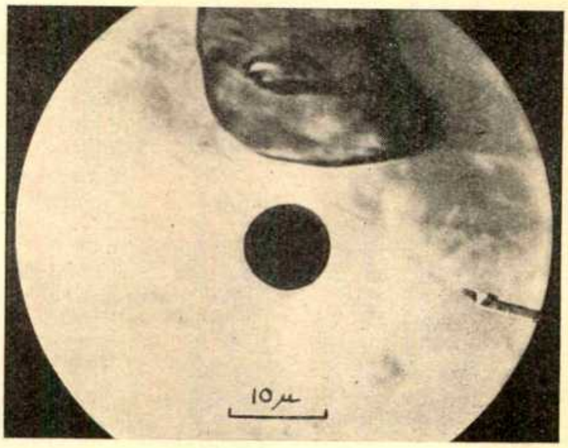

Fig. 2

The value to migratory problems of the evidence from this moth depends on where it picked up the particle—in Africa or in more northerly latitudes. Meteorological data suggest that the main radioactive cloud travelled eastward in a fairly narrow cone. Rises in levels of activity observed in western Europe indicate arrival of the cloud at the end of February (26–29) after the first global circuit. At this age, it is unlikely that 9-μ particles would still be present. A possibility would be of a small, highly active particle, airborne for two weeks, and afterwards attached to a 9-μ particle of natural dust. This could have happened anywhere over Europe. However, the particle observed was not a resolvable aggregate and, as stated above, was typical of particles originating in the high temperature of the explosion. There is also the possibility of pockets of contaminated tropical air passing over southern Europe and depositing radioactive particles in the period immediately after the explosion. We have no definite evidence to support this beyond a slight rise in southern Italy on February 22–23. However, even if this did occur, large particles such as that on this *N. noctuella* would be very rare, whereas they could be quite abundant in the area of immediate deposition in the Sahara. I believe, therefore, that there must be a very strong probability that this moth acquired its radioactive particle before leaving Africa on its journey northward.

Conclusions. The evidence provided suggests, but not conclusively, that insects as small as the moth *N. noctuella* can cover a distance of approximately 1,500 miles in the course of their short lives.

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Influence of Hypothermia on the Survival of Mice after Large Doses of X-Radiation

It is known that hypothermia applied after irradiation prolongs the survival of frogs[1,2] and hibernating animals[3], and slows down destructive processes in the hæmatopoietic tissue and blood[3,4]. In our previous work we showed that prolonged hypothermia at 22–25° C., lasting 6–48 hr., after irradiation also slows down destructive changes in the blood, spleen and bone marrow[5,6]. After the ending of hypothermia and reaching the normal body temperature these changes, however, develop even more intensely than in controls subjected to irradiation only and the mean survival-time is shorter. After doses up to 15,000 r. animals survive for more than 3 days after irradiation[7] and do not tolerate such prolonged hypothermia. Therefore investigations of the survival-time after irradiation in homoiothermic animals under hypothermia can be carried out only after large doses of irradiation where the control animals die a few hours following irradiation, which is a well-tolerated duration of hypothermia.

In our experiments white male mice were used weighing 20–25 gm., which were divided into four groups: irradiated controls without hypothermia (Table 1, V), mice with intraperitoneal injections of 'Largactil' and 'Phénergan' ('Specia') solutions at 0·2 mgm./10 gm. body-weight which were subjected immediately after irradiation to hypothermia, that is, a body temperature of 20–24° C. measured by thermocouple in the rectum (Table 1, III, VII—artificial hypothermia), mice subjected to hypothermia in ice without the lytic mixture and maintained up to death at a body temperature of 20–24° C. (Table 1, IV, VIII—hypothermia) and finally mice with luminal sodium anæsthesia (Table 1, II) and thiopentone sodium anæsthesia (Table 1, VI). We irradiated four mice at a time, one from each group, in a plexit box on a turning-table (two rotations per min.) which ensures homogeneity and uniform X-ray dosage. Mice of groups I–IV were subjected to whole-body irradiation with 40,000 r., groups V–VIII 45,000 r. (180 kV., 15 m.amp., without filter, target distance 16 cm., dose-rate 1,000 r./min.).

Table 1. STATISTICAL EVALUATION AS COMPARED WITH CONTROLS

Group	No. of animals	Mean survival time (hr.)	Minimum and maximum survival time (hr.)
	40,000 r.		
I Control	24	2·02 ± 0·18	0·35– 3·85
II Luminal sodium $t = 27$–$30°$ C.	20	3·72 ± 0·31 $P = 0·001$	1·20–6·30
III Artificial hypothermia $t = 20$–$24°$ C.	20	6·57 ± 0·55 $P < 0·001$	1·25–12·35
IV Hypothermia $t = 20$–$24°$ C.	24	6·06 ± 0·57 $P < 0·001$	0·90–13·35
	45,000 r.		
V Control	15	1·29 ± 0·15	0·60–2·50
VI Sodium thiopentone $t = 34$–$35°$ C.	17	1·10 ± 0·11 not significant	0·35–1·95
VII Artificial hypothermia $t = 20$–$24°$ C.	15	3·87 ± 0·37 $P < 0·001$	1·65–7·50
VIII Hypothermia $t = 20$–$24°$ C.	17	3·60 ± 0·47 $P < 0·001$	1·15–7·20

In a special experiment we confirmed the finding[7] that the mean survival-time of mice exposed to doses of 15,000–50,000 r. decreases exponentially with the rising dose of irradiation and is thus a suitable criterion for our experiments.

The mean survival-time of mice after irradiation is recorded in Table 1. Hypothermia prolongs with statistical significance the mean survival-time of animals and reduces the effective dose of radiation from 40,000 r. to about 35,000 r. and from 45,000 r. to about 38,000 r. This effect can be ascribed to two mechanisms: hypothermia which slows down the development of pathophysiological processes after irradiation and the associated inhibition of the central nervous system which leads to a mitigation of symptoms described in the literature after these large doses of irradiation, to a reduction of the hyperexcitability and to a delay of the convulsive state. The inhibitory effect of hypothermia of 20–24° C. corresponded approximately to the effect of thiopentone, after which we did not record an increase in the survival time. Sodium luminal anæsthesia had a protective effect, but reduced at the same time the body temperature to 27–30° C., and therefore we cannot ascribe the longer period of survival entirely to the inhibition of the central nervous system. It is more probable that the main part is played by hypothermia and the associated inhibition of the central nervous system enhances the protective effect. This may explain also the somewhat more favourable results obtained with hypothermia when combined with the administration of the lytic mixture than with hypothermia alone.

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Theoretical Considerations in the Ultra-violet Irradiation of Micro-organisms

Morowitz[1] has derived equations relating survival ratio (N/N_0) for a given species of micro-organism to the ultra-violet quantum density (B_0) at the surface of an irradiated volume of liquid of absorbance α and uniform depth L. For a micro-organism of 'target area' S, the equation for a perfectly mixed volume of liquid is:

$$\frac{N}{N_0} = e^{-SB_0 \frac{1 - e^{-aL}}{aL}} \qquad (1)$$

and for a stratified, or completely unmixed liquid:

$$\frac{N}{N_0} = \frac{1}{\alpha L} \int_{SB_0 e^{-aL}}^{SB_0} \frac{e^{-u}}{u} \, \mathrm{d}u \qquad (2)$$

For experiments with thin films of liquid supported on polished aluminium or other highly reflective surfaces, I have found it necessary to modify the Morowitz equations to allow for the contribution of light which is reflected back into the liquid. When the reflectance of the supporting surface is R, equation 1 becomes:

$$\frac{N}{N_0} = e^{-SB_0 \frac{1 - e^{-aL} + Re^{-2aL}(e^{aL} - 1)}{aL}} \qquad (3)$$

and equation 2 becomes:

$$\frac{N}{N_0} = \frac{1}{\alpha L} \int_{e^{-aL}}^{1} \frac{e^{-SB_0\left(u + \frac{Re^{-2aL}}{u}\right)}}{u} \, \mathrm{d}u \qquad (4)$$

For films of liquid thin enough or transparent enough, the degree of mixing is theoretically unimportant, since equations 3 and 4 (as well as 1 and 2) converge for very low values of αL. To determine the limiting values of film transmittance (e^{-aL}) above which mixing may be ignored as a variable, I computed the deviation between equations 3 and 4 for $0 < e^{-aL} < 1$ and $0 < R < 1$, using the IBM 650 computer with the assistance of Mr. John H. Witmer. For survival ratios down to 10^{-6}, the calculated deviation was found to be less than 12·6 per cent (one-tenth of a logarithmic unit of infectivity) when film transmittance was above the limiting values shown in Table 1. This deviation is negligible in comparison with the usual errors in measuring microbiological activity.

Table 1. Values of Film Transmittance (e^{-aL}) above which Mixing of the Liquid during Irradiation does not Appreciably influence the Predicted survival Ratio

R	e^{-aL}	R	e^{-aL}
0	0·84	0·5	0·69
0·1	0·81	0·6	0·66
0·2	0·78	0·7	0·63
0·3	0·75	0·8	0·60
0·4	0·72	0·9	0·57

For film transmittances greater than the limiting values, therefore, mixing of the liquid during irradiation seems to be unnecessary, and equation 3 adequately describes the predicted course of inactivation whether the liquid is mixed or not.

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BIOLOGY

Water-Conduction in the Stems of Certain Conifers

In a laboratory study of the rate of flow of water through sapwood samples of *Pseudotsuga taxifolia* (Poir.) Britt., *Pinus nigra* Arnold, and *P. radiata* D. Don., the effective water-conducting areas were determined by using aqueous solutions of various dyes. It was observed that water-flow in the direction of the grain was restricted to the early wood, and that the late wood only became coloured, if at all, by later diffusion of dye, especially along wood rays. When acid fuchsin was used the wood became almost uniformly coloured due to the ready diffusion of this dye across the late wood, but when safranin was used the late wood remained unstained.

The late wood of all three species appeared to be drier than the early wood, and showed up as lighter coloured streaks on the radial longitudinal surfaces of fresh sapwood. To examine this condition in detail, radial strips of wood, 1 cm. square, cut from freshly felled trees, were dissected into early wood and late wood samples and weighed immediately after dissection. Though complete separation was difficult, the early wood proved to be 95 per cent saturated with water, whereas the late wood, apart from the outermost growth layer, was only 65–70 per cent saturated.

Safranin dye injected into living trees was also shown to move along the early wood, the late wood only becoming stained some distance behind the limits of dye movement. As in the laboratory experiments, acid fuchsin passed into the late wood so rapidly that the effect was less apparent.

These phenomena have been observed from June (mid-winter) until February, and, although resin-flow in the two pines interfered with dye-injection experiments during the spring, laboratory studies of water-flow and percentage saturation gave similar results at all times.

In the coniferous species examined, it therefore appears that the late wood is not an effective water-conducting tissue owing to its low degree of saturation with water. It is possible that the structure of the bordered pits in late wood prevents effective aspiration when an air bubble forms in a tracheid, so that the conducting columns soon break down. Whatever the cause, the implications are of interest when considering water conduction in the living stems, and also for wood preservation by diffusion processes.

In a previous communication[1], it was suggested that the percentage of pits aspirated in the early wood of the inner sapwood was related to the degree of saturation of the wood with water. But, as the percentage saturation was determined for the late wood and early wood together, it is obvious from the present work that this was a chance relationship, and some other cause must be sought to explain the progressive aspiration of bordered pits from the outer to the inner sapwood.

Finally, in considering water-flow in these trees, it seems that we must recognize the possibility that continuity between the transpiring leaves and the roots is only possible along those growth-layers penetrated by the traces of living needles. Growth-layers interior to these are isolated by non-conducting bands of late wood, and although these are crossed by ray tracheids, observations of the flow of dye through radial sections of the stem have shown that the dye moves on a broad front, and does not pass more freely along rows of ray tracheids than it does along the ray parenchyma. This apparent discontinuity of the late wood therefore implies that, in the inner sapwood, the movement of water by cohesion takes place along a series of separate concentric paths bridged only by the osmotic forces in the living cells of wood rays.

It is intended to publish these results in greater detail elsewhere.

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Phenomic Delay in Transduction of *Salmonella typhimurium* Cell

Transduction has been demonstrated in the genus *Salmonella* by Zinder and Lederberg[1]. The individual markers were induced independently of each other according to the selective medium used. Even in cultivation conditions with two sugars, invariably only one of them was fermented by the individual transduced colonies.

In my experiments I studied the mechanism of transduction of galactose fermentation. Basically, I wanted to determine whether the given substrate acts only as a selection factor in transduction, or participates also in the mechanism of transduction. I used strains of *Salmonella typhimurium*, LT_2 (Fen+, Ty+, Tr+; Gal+, Xyl+) and SW_{351} (Fen−, Ty−, Tr−; Gal−, Xyl−), described by Zinder and Lederberg[1] and kindly sent to me by them. A constitutive galactozymase enzymatic system was found. Also, the transduction occurs even without the presence of a specific substrate. It was found, however, that the transduced cells multiply either very slowly under these experimental conditions without the galactose or do not multiply at all.

I tried, therefore, in further experiments to find out whether the presence of this fairly high number of recipient cells (about 10^9) has no inhibitory effect on the growth of newly started transduced cells. Table 1 shows the results of a typical dilution experiment[2]. The suspension of the known number of cells, obtained by washing a 48-hr. SW_{351} strain cultured on complete agar[3], was mixed with the filtrate of the transducing phage in ratio 1 : 1 and exposed for 30 min. at 37° C. The cell suspension with the adsorbed phage particles was then successively diluted with the complete medium and always two eosin methylene blue agars from each dilution were inoculated either without or with a 1 per cent solution of galactose. The number of colonies fermenting galactose on the eosin methylene blue agars with the galactose indicates the number of inoculated cells with the adsorbed phage. After 24- or 48-hr. growth the film of bacteria from the eosin methylene blue agars without the galactose was washed off in saline, the suspension was diluted to 2 ml. and the total number of cells as well as the number of transduced galactose-fermenting cells were determined. The number of cell-divisions in the culture was estimated from the amount of recipient cells inoculated on the eosin methylene blue agar without galactose and the number of cells grown after 24 or 48 hr. (ref. 4). I determined then, in the same way, the number of divisions of transduced cells from the amount of galactose-fermenting cells on eosin methylene blue agars inoculated with a diluted mixture of cells and phage particles, and from the amount of galactose-fermenting colonies after 24 or 48 hr. growth.

Table 1 indicates that the increased number of divisions of all cells is also connected with a higher number of divisions of the transduced cells. It can, therefore, be concluded that no specific substrate, which acts only as a selection factor, is needed for the transduction. Under the given experimental conditions, that is, without the selection factor and in the presence of a great number of inoculated cells, the growth of the transduced cells is inhibited.

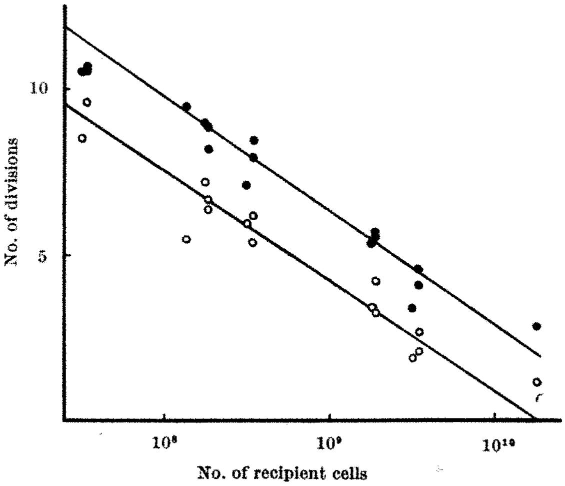

Fig. 1. The number of divisions of recipient (—●—) and transduced (—○—) cells

Table 1. INFLUENCE OF INITIAL NUMBER OF CELLS PLATED ON THE GROWTH OF THE RECIPIENT AND TRANSDUCED CELLS

Exp. No.	No. of cells		No. of divisions	No. of inoculated cells capable of transduction	No. of Gal + cells after growth*	No. of divisions of Gal + cells	No. of Gal + cells to colony
	inoculated	after growth*					
1	$2\cdot5 \times 10^{10}$	$1\cdot94 \times 10^{11}$	2·9	252	600	1·2	2·3
	$5\cdot0 \times 10^{9}$	$5\cdot2 \times 10^{10}$	3·4	237	880	1·9	3·7
	$2\cdot5 \times 10^{9}$	$9\cdot8 \times 10^{10}$	5·3	116	1,200	3·4	10·3
	$5\cdot0 \times 10^{8}$	$6\cdot8 \times 10^{10}$	7·1	26	1,700	6·0	65·4
	$2\cdot5 \times 10^{8}$	$1\cdot2 \times 10^{11}$	9·0	14	2,080	7·2	145·0
	$5\cdot0 \times 10^{7}$	$7\cdot2 \times 10^{10}$	10·5	2	700	8·5	350·0
2	$5\cdot4 \times 10^{9}$	$1\cdot3 \times 10^{11}$	4·6	212	920	2·1	4·3
	$2\cdot7 \times 10^{9}$	$1\cdot4 \times 10^{11}$	5·7	106	1,880	4·2	17·7
	$5\cdot4 \times 10^{8}$	$1\cdot4 \times 10^{11}$	8·0	21	900	5·4	42·8
	$2\cdot7 \times 10^{8}$	$1\cdot2 \times 10^{11}$	8·9	10	860	6·4	86·0
	$1\cdot3 \times 10^{8}$	$9\cdot6 \times 10^{10}$	9·5	5	220	5·5	44·0
	$5\cdot4 \times 10^{7}$	$8\cdot8 \times 10^{10}$	10·6	2	1,600	9·6	800·0

* 24-hr. and 48-hr. growth for experiments 1 and 2 respectively.

Table 1 shows further that the number of divisions of transduced cells has on an average two divisions less than the number of divisions of all recipient cells (Fig. 1).

I studied, therefore, this phase of the delayed phenotypic expression[5] to find out whether the problem in question is a matter of change of the genotype (changes in the deoxyribonucleic acid (DNA)), or of the phenotype expression of the changed genotype (synthesis of certain building blocks of new enzymes). Chloramphenicol was used as the inhibitor of the proteosynthesis. This does not inhibit the synthesis of the genetically functioning DNA. The cell suspension with the adsorbed phage was diluted at a ratio of 1 : 50 by a complete medium and cultivated for 6 hr. at 37° C. Chloramphenicol (final concentration 20 μgm./ml.) was added at 0, 2, 4 and 6 hr. cultivation and afterwards the cells were centrifuged and washed twice with saline. Thereafter the number of generations of recipient and transduced cells was determined as in previous experiments. The results are given in Table 2.

Table 2. INFLUENCE OF CHLORAMPHENICOL ON THE PHASE OF THE DELAYED PHENOTYPIC EXPRESSION

Cultivation at 37° C. (hr.)	Choramphenicol added after hr. of growth	No. of divisions before adding chloramphenicol	No. of divisions of recipient cells (*a*)	No. of divisions of transduced cells (*b*)	Difference in division (*a*−*b*)
—*	—	0	11·5 ; 11·3	8·1 ; 7·3	3·7
6	0	0	12·5 ; 12·2	11·4 ; 10·5	1·4
6	2	1·2	11·4 ; 11·9	10·7 ; 10·4	1·1
6	4	5·5	7·6 ; 6·8	7·8 ; 6·7	0
6	6	5·8	7·3 ; 7·0	6·8 ; 6·8	0·3

* Control sample without chloramphenicol and without cell division.

It was found that a complete compensation of the number of recipient and transduced cells occurs after the fourth hour of cultivation in the complete medium without chloramphenicol, where the five generations occur. The presence of chloramphenicol shortens the phase of delayed phenotypic expression, that is, certain processes are accomplished which are not inhibited by chloramphenicol and are part of the changes of genotype of the recipient cell. At the same time, even with the presence of chloramphenicol, there is a certain delay in the number of generations, which is the growth and proteosynthetic phase, inhibited by chloramphenicol, and part of the expression of phenotype.

The problem of the phase of the delayed phenotypic expression will be subjected to further biochemical examination.

I wish to express my appreciation to Academician I. Málek for his valuable suggestions and encouragement.

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Acquisition of Graft versus Host Tolerance

RECENTLY, Simonsen[1] and Siskind, Leonard and Thomas[2] have reported attempts to produce 'runt disease' in neo-natal mice by the passage of the cellular chimera established in lymphoid organs of infant mice following the injection of spleen cells obtained from adult mice. As Simonsen and Jensen[3] had previously noted that splenic enlargement was a regular and early symptom in affected animals, this phenomenon was used by Simonsen to assess the graft-versus-host response. He concluded that the chimeric cells fail to produce runt disease on passage. This finding appears to conflict with the results described by Siskind and his colleagues, who observed that infant recipients of the appropriate strain not only showed similar symptoms on passage of the chimeric cells, but also that the runt syndrome observed in the passage animals was symptomatically more severe. The analysis of the runt syndrome carried out in this Laboratory supports and extends the findings described by Simonsen.

My work has generally been confined to the production of the runt syndrome in mice following the intravenous injection of *C57B*1 adult spleen cells into *C3H* neo-nates (within 24 hr. of birth), and the reciprocal situation which is obtained when *C3H* adult spleen cells are injected into *C57B*1 neo-nates. When 10 × 10^6 or more adult *C57B*1 spleen cells are injected intravenously into *C3H* neo-nates, all recipients become acutely affected and die within three weeks of injection. The injection of 10 × 10^6 *C3H* adult spleen cells into *C57B*1 infants produces the less-severe symptoms of chronic runt disease, with about 50 per cent of the injected animals surviving two or more months. Passage was made by removal of spleens from the affected mice 6–21 days after inoculation. Usually 10 × 10^6 dissociated cells were passaged intravenously in neo-nates. In these passage experiments runting occurred in only a small proportion of mice or was completely absent. Tests for chimeric cells were made at the time of passage

Table 1. INCIDENCE OF RUNTING AND CHIMERISM ON PASSAGE OF SPLEEN CELLS OBTAINED FROM ACUTE AND CHRONIC RUNTS

System	Passage	Percentage runting	Chimerism (chimeric cells detected in X nucleated spleen cells)
(1) Spleen cells obtained from normal donors.			
(*a*) *C57* adult spleen cells injected into *C3H* neo-nates	Primary induction	100 (11/11)	$X = 20 \times 10^6$
	1st passage	25 (2/8)	$X = 20 \times 10^6$
	2nd ,,	0 (0/8)	$X = 20 \times 10^6$
(*b*) *C3H* adult spleen cells injected into *C57B*1 neo-nates	Primary induction	100 (39/39)	Not tested
	1st passage	0 (0/40)	$X = 20 \times 10^6$
(2) Spleen cells obtained from immunized donors			
(*a*) Pre-sensitization by skin graft of recipient strain. *C3H* adult spleen cells injected into *C57B*1 neo-nates	Primary induction	100 (22/22)	*c.* 10 per cent n.c.* chimeric
	1st passage	60 (8/13)	*c.* 5 per cent n.c.* chimeric
	2nd ,,	0 (0/15)	*c.* 5 per cent n.c.* chimeric
(*b*) Pre-immunization by injection of donors with recipient strain spleen cells. *C3H* adult spleen cells injected into *C57B*1 neo-nates	Primary induction	100 (30/30)	$X = 10 \times 10^6$
	1st passage	0 (0/18)	$X = 10 \times 10^6$

* Percentage of nucleated spleen cells estimated by titration of the chimera to be of donor strain origin.

using Billingham and Brent's[4] technique, in which the presence of donor-type cells is shown by the accelerated rejection of a donor skin graft in 10-week mice inoculated 7 days previously with the spleen cells in question. In some experiments graded doses were used in an attempt to assess the proportion of skin cells.

Tests for chimeric cells were also made on spleens obtained from the unaffected first or second passage recipients (Table 1). These experiments demonstrate the persistence in the passage animals of chimeric cells which fail to elaborate a response against the host. It is reasonable to conclude, therefore, that the chimeric cells have become tolerant of host antigens during passage. This conclusion is confirmed and extended by work on the passage of spleen cells obtained from donor mice which were immunized with recipient strain spleen cells, and donors which had been pre-sensitized with an orthotopic skin graft of the recipient strain. Again the incidence of runt disease was either greatly reduced on the first passage and absent on second passage, or the infant recipients completely failed to show any signs on first passage of the chimeric cells. The presence of the chimeric donor cells was again detected in the spleens of the normal passage animals (Table 1).

On several occasions evidence of casual infection, presumably by a virus, was obtained on passage of spleen and liver obtained from chronic runts six weeks following injection of homologous spleen cells. This was not seen when neo-nates were injected with isologous spleen cells obtained from normal adult mice. The presumed virus produces a progressive and fatal disease in infant mice which closely resembles acute runt disease. It produces no symptoms in adult mice. Histologically, gross destruction of liver cells is seen in the affected mice. The cellular infiltration observed in the virus-infected livers is polymorphonuclear in nature. The liver lesion produced by the virus may therefore be readily distinguished from the liver lesion produced in immunologically affected animals by graft-versus-host reaction, as this latter lesion is characteristically associated with a lymphocytic infiltration. It is probable that latent colony infections ultimately bring about the death of the debilitated chronic runts, and consequently passage of organs of such animals is liable to contamination of the cell chimeras with latent infection.

A fuller account of this work will be given elsewhere.

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Adipokinetic Activity of Synthetic Peptides Related to Corticotropin

SYNTHETIC peptides prepared by Hofmann and associates[1] containing 8, 10 or 13 amino-acids identical in sequence with the N-terminal portion of corticotropin were tested for adipokinetic activity because corticotropin was known to have this activity[2–4]. The tridecapeptides caused the release of free fatty acids by adipose tissue; the decapeptides were also active but less potent, but the octapeptide had no effect in the amounts tested.

The synthetic peptides were:

	N-terminal	1	2	3	4	5	6	7	8	9	10	11	12	13
I		ser	tyr	ser	met	gluta	his	phe	arg					
II	N-acetyl	ser	tyr	ser	met	gluta	his	phe	arg	try	gly			
III		ser	tyr	ser	met	glu	his	phe	arg	try	gly			
IV	N-acetyl	ser	tyr	ser	met	gluta	his	phe	arg	try	gly	lys	pro	valamide.
V		ser	tyr	ser	met	glu	his	phe	arg	try	gly	N-formyl lys	pro	valamide.

For comparison, the structure of the amino-terminal portion of corticotropin and the total structure of α-melanocyte-stimulating hormone are presented:

		1	2	3	4	5	6	7	8	9	10	11	12	13	
ACTH		ser	tyr	ser	met	glu	his	phe	arg	try	gly	lys	pro	val	. . . 39.
α-MSH	N-acetyl	ser	tyr	ser	met	glu	his	phe	arg	try	gly	lys	pro	valamide	

ACTH, Adrenocorticotrophic hormone; MSH, melanocyte-stimulating hormone

When added to rat epididymal adipose tissue incubated in 1·0 ml. Krebs–Ringer phosphate–5 per cent defatted albumin, 10 μgm. of IV or V stimulated the production of free fatty acids (Table 1). A smaller but significant response was seen with 1 μgm. II was active at a dose of 100 μgm., III was probably active at that dose, and I was inactive. The minimal effective dose of corticotropin is about 0·001 μgm.[4].

In contrast to the weak effect of the peptides relative to corticotropin on rat tissue, the potency of IV and of V was similar to that of corticotropin

Table 1. Effect of peptides on production of free fatty acids by pieces of rat epididymal adipose tissue (approximately 100 mgm.) in 1·0 ml. Krebs–Ringer phosphate–5 per cent albumin, *p*H 7·4, incubated in Dubnoff shaker for 3 hr. at 37°. The control and experimental samples of adipose tissue were obtained from the same rats. Medium and tissue were extracted together at end of incubation and titrated

Test substance	No. of samples	Dose (μgm./ml.)	Free fatty acid after incubation (μequiv./gm./hr.)	*S.E.*	*P* value
Control	17	—	3·3	0·49	
I	17	100	3·2	0·53	—
Control	17	—	3·1	0·50	
II	17	100	5·8	0·91	≈ 0·01
Control	17	—	3·3	0·51	
III	17	100	4·4	0·57	—*
Control	17	—	2·1	0·33	
IV	17	10	6·1	0·79	< 0·001
Control	17	—	2·1	0·33	
V	17	10	5·3	0·62	< 0·001

* Difference is significant if *P* value calculated from differences of matched pairs. Values presented in Table 1 were obtained from formula

$$t = \frac{\text{difference of means}}{S.D.\text{ of combined groups}} \sqrt{\frac{N_1 N_2}{N_1 + N_2}}$$

Table 2. Free fatty acids after incubation of pieces of rabbit perirenal adipose tissue. Conditions were same as in Table 1. Number of samples in parentheses

Test substance	Free fatty acid (μequiv./gm./hr.) after incubation with indicated dose (μgm./ml.)					
	0	0·01	0·03	0·1	10	30
Control	0·95 (11) ± 0·42 (*S.D.*)	— —	— —	— —	— —	— —
I	—	—	—	—	—	0·71 (8)
II	—	—	—	0·78 (4)	9·3 (4)	
III	—	—	—	—	8·7 (4)	—
IV	—	2·4 (9)	8·0 (4)	10·4 (5)	—	—
V	—	1·5 (5)	4·4 (4)	9·4 (6)	—	—

when tested either on isolated rabbit adipose tissue or in the intact rabbit. The output of fatty acids *in vitro* was increased by 0·01 μgm. of IV, and by 0·03 μgm. of V. II was inactive at 0·1 μgm., but both II and III were strongly active at 10 μgm., while I was inert at 30 μgm. (Table 2). When injected subcutaneously in 2–3 kgm. male rabbits, 10 μgm. of IV or 30 μgm. of V was sufficient to increase severalfold the plasma free fatty acids measured 1½ hr. later. 300 μgm. of II, the largest dose tested, was ineffective (Table 3).

The adipokinetic property of corticotropin is thus first encountered when the amino-terminal octapeptide is elongated to the decapeptide, and the potency increases with the addition of the next three amino-acids. The increased potency of the tridecapeptide was seen both in the rabbit, in which the tridecapeptide was as active as corticotropin, and in the rat in which it was only 1/1,000 as active. N-acetylation of the terminal serine did not lower the adipokinetic activity of the peptides and may have increased it slightly. By comparison, terminal acetylation of corticotropin has been reported to decrease corticotropic activity to less than 10 per cent, while enhancing melanophore-expanding activity[5]. This first demonstration of fat mobilization caused by synthetic peptides may serve as a guide to the structure of compounds controlling this aspect of fat metabolism and may facilitate an analysis of the interrelated structural requirements for adipokinetic, corticotropic and melanophore-expanding activity.

Table 3. Free fatty acids of plasma 1½ hr. after subcutaneous injections of test substance in fed male rabbits weighing 2–3 kgm. Number of tests in parentheses

Test substance	No injection	Negative tests	Plasma free fatty acids (μequiv./l.) after indicated dose (μgm.)					
			3	10	30	100	300	1,000
None	559 (7) ± 153 (*S.D.*)	543 (52) ± 170 (*S.D.*)						
II							495 (3)	
IV			231 (2)	1,839 (4)	2,034 (4)	3,600 (1)	2,280 (1)	3,480 (1)
V				586 (2)	1,041 (2)	1,497 (2)	2,633 (2)	3,420 (1)

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Opal in the Animal Body

Recent work on opal-phytoliths[1–4] has led to searches for these microscopic bodies of amorphous silica in the body of a sheep. This is a typically herbivorous animal that ingests significant quantities of silica as integral parts of its plant food.

The food and the rumen contents of a sheep on a diet of oaten hay have been examined both before and after destruction of organic matter[5]. By the destruction of organic matter with nitric and perchloric acids, insoluble residues were obtained which consist largely of opal-phytoliths. Accompanying the opal-phytoliths is a small proportion of other particles including several mineral species, diatom frustules, silicisponge spicules, and fragments and microspheres of artificial substances. These particles are mechanically incorporated with the food and ingested by the sheep; they are more common in the ventral than in the dorsal regions of the rumen. It is proposed to name them allothigenes, and it is intended that this term should include all particles which are generated outside the animal body and which are taken into the body either by ingestion or inhalation.

The amounts of allothigenic material ingested by sheep may be substantial. It is estimated that a total of 10 gm. was present in the rumen of the sheep fed on oaten hay; opal-phytoliths made up 95 per cent of this amount. In some plants, for example *Lolium rigidum*, the content of opal-phytoliths can be twice as high as in oats, and the rumen of a sheep feeding on such plants can be expected to contain 20 gm. of allothigenic material. From examinations of fæcal material it is evident that the allothigenes are inert and that most of them are excreted after passing along the alimentary tract.

The knowledge that substantial quantities of allothigenes are presented to the epithelial surfaces of the alimentary tract led to a search for them in the lymphatic system[6]. The following lymph nodes were taken from slaughtered sheep: prescapular, mediastinal, mesenteric, popliteal, precrural and bronchial. Insoluble residues obtained by digestion with nitric and perchloric acids revealed opal-phytoliths and several mineral species in every node. The presence of these allothigenes was confirmed in sections of prescapular and mesenteric nodes.

The mechanism of entry of allothigenes into the lymphatic system is not known. It is interesting, however, to note that much of the pathway of absorption of particles of fat from the intestinal mucosal surface to the lacteals is extra-cellular. Such a pathway may well hold for the allothigenes. Other factors contributing to their entry would undoubtedly be their size, shape and hardness combined with the various movements of the intestine, such as vascular pulsations, peristalsis, segmentation, and the pumping action of the mucosal villi.

Entry of opal-phytoliths and other minerals into the tissues of the animal subsequent to ingestion has not been recognized before. On the other hand, it is well known that sheep ingest appreciable quantities of soluble silica, most of which is absorbed and finally excreted in the urine. It is less well known that the absorbed silica may be precipitated in various parts of the body to form opal-zooliths. Siliceous uroliths provide an example of this process. This led us to examine specimens obtained from a ram which had died of urolithiasis[8]. X-ray and microscopic techniques showed that the uroliths of this ram were opaline silica, like those found in steers[9]. The most striking fact about the uroliths of the ram was the presence within them of opal-phytoliths. It seems that they sometimes act as nuclei and initiate the precipitation of silica. These allothigenes must have entered the blood stream and finally penetrated the glomerulus. The conclusion that allothigenes can enter the blood-stream is supported by the fact that birefringent allothigenes have been found in the blood and organs other than the lungs of humans, having found their way into the general circulation from the lungs[10].

In view of their inertness, size, shape and hardness it is possible that the allothigenes damage tissues and impair function in more ways than has yet been recognized. Opal-phytoliths are a major cause of wear in teeth[11], and they affect the depth of the stratum corneum of the rumen[5].

The insoluble residues of lymph nodes which were examined for allothigenes also showed evidence of precipitation of silica on a micro scale. Several types of corpuscles including phagocytes, lymphocytes, and red blood corpuscles were observed to have been mineralized by amorphous silica and by silica plus magnesium plus phosphorus, thus giving rise to micro-zooliths. That this process might sometimes occur on quite a considerable scale is evident from the observation that one million mineralized corpuscles were estimated to be present in one prescapular lymph node. There may be a close parallel between this mineralization and the 'mummifying' effect which silica has been found to have on phagocytes which ingest it[12]. In contradistinction to the introduced allothigenes it is proposed to use the term authigenes for zooliths, either macro- or micro-, which are produced by secretion within the body.

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CYTOLOGY

The Pachytene Stage in Mammalian Oocytes

Several chromosomal abnormalities have recently been described in human cytogenetics, some of them probably directly and causally related to certain phenotypical effects. All this work was carried out using chromosomes of the mitotic metaphase, mostly from cultures of various cells. The exact identification of any single chromosome is not possible. All 42 autosomes and a pair of sex chromosomes are divided into four or five morphological classes using the length of arms and the position of the centromere as a landmark of identification. The difficulties may be illustrated by the fact that the sex of an individual is determined as male if there is an uneven number of small chromosomes. There is a great need for a more precise method of identification by which individual chromosomes can be recognized.

The study of chromosome morphology in meiotic prophase may perhaps open up some possibility in this direction. Mapping of human chromosomes in the pachytene stage of the spermatocyte is probably only a matter of time, and it is probable that in the near future there will be available a satisfactory pachytene map of human chromosomes from the spermatocytes.

It has been known for some time that meiotic prophase in female mammals starts in the embryonic ovary and stops after the stage of pachytene has been reached. This is followed by a diffuse stage called dictyotene in which the oocytes remain until the time of ovulation.

More detailed investigation on mouse material has shown that meiotic prophase in the oocytes starts

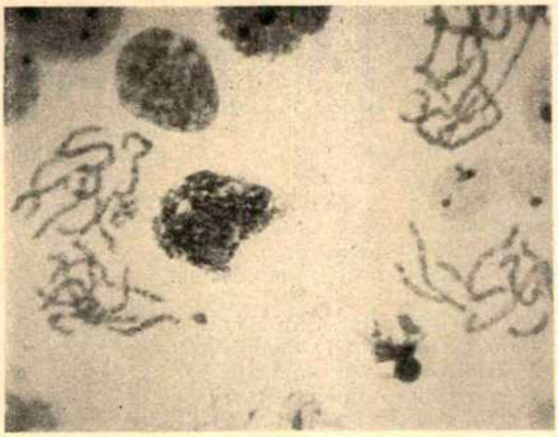

Fig. 1

at about the fifteenth day of pregnancy, when the leptotene stage appears in the nuclei of the young oocytes. This is followed by zygotene and finally by pachytene, which is the best stage for cytological identification of individual chromosomes. A few days after birth all oocytes are in the dictyotene stage and all possibility of chromosomes being identifiable has vanished. Comparison of data obtained here with those (unpublished) of Drs. K. Borum and H. Peters (Finsen Laboratory, Copenhagen, personal communication) indicates that the time of appearance of leptotene and of dictyotene depends on genetic constitution.

Embryonic ovaries taken at about the fourteenth day of pregnancy or any day later can be cultured in a synthetic mixture (*M*-199) for a few days (up to 48 hr. in excellent condition—later the stainability deteriorates). The number of oocytes in the pachytene stage probably increases in the culture. The accompanying photomicrograph shows pachytene in oocytes removed from a 14 days old mouse embryo and kept in the medium at 37° C. for 48 hr.

Although there are great obstacles and difficulties in the application of this method to human material, it is perhaps worth while to keep its potential possibilities in mind.

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Absorbance of Feulgen-stained Bull Sperm

IN earlier communications[1,2] it was suggested that both the absorbance and the geometrical dimensions of Feulgen-stained somatic cell nuclei were dependent variables of a common, independent variable, which was considered to be the 'growth process'.

As the genetic complement of a nucleus replicates itself between two consecutive divisions, and as replication implies a strictly orderly arrangement of the participating molecules both in time and space, the growth process can be described as being a process by which molecules are brought from a state of less to a state of higher orderliness. Accordingly the absorbance of Feulgen stained nuclei can be interpreted as being a measure of the ratio between the number of molecules in the states of orderliness and disorder[1]. In the case of somatic cell nuclei the numerical value of this ratio will depend on the type of arrangement of the nuclear genetic complement, the rate of synthesis and the interphase age, while in the case of spermatozoa, which are haploid 'end products', the absorbance is only dependent on the arrangement of the genetic complement.

The modal distribution of measured absorbances from a population of spermatozoa is accordingly interpreted as being not the result of a Gaussian distribution of measuring errors, but as the result of different arrangements of the genetic complement. The mode indicates therefore that either the majority of the spermatozoa possesses identical arrangements of the genetic complement or that the differences in arrangement are so slight as to give rise to differences in absorbance too small to be detectable with the applied measuring technique, while outlying absorbance values indicate differences in arrangement sufficiently large to be measurable.

The feasibility of this interpretation seems to be corroborated by the following results: the distribution of identically and simultaneously treated Feulgen-stained sperm heads from three Ayrshire dairy bulls are shown in Fig. 1, from which it is evident that the three distributions are different. The absorbances are lowest for bull *A*, which is 9·5 years

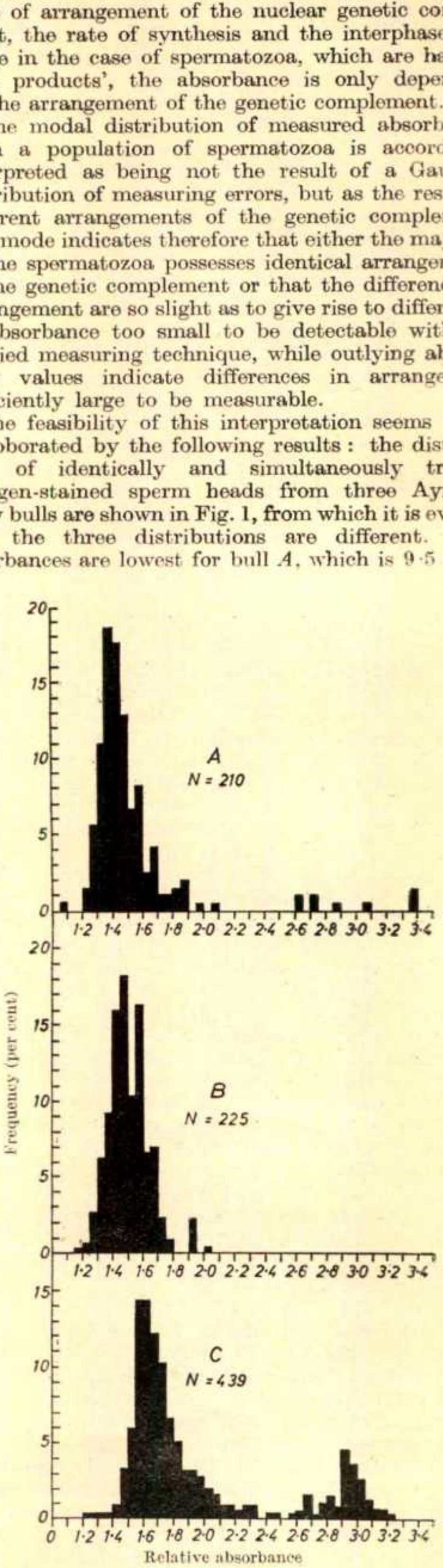

Fig. 1

old and from which 41 daughters averaged 6,691 lb. of milk; highest for bull *C*, which is 7 years old and from which 13 daughters averaged 10,052 lb. of milk; and intermediate for bull *B*, which is 5 years old and from which 13 daughters averaged 7,596 lb. of milk.

Although a correlation between the distribution of absorbances and milk yield in the offspring exists, it should be emphasized that it is not claimed that a given distribution of absorbances is characteristic for a given gene, or genes, or for a phenotypic characteristic, but it seems that the distribution of absorbances might give a relative measurement which possibly can be used as a screening test. Thus assuming that the gene(s) for high milk-yield in the offspring were present in spermatozoa with the genetic complement arranged in such a way as to give rise to absorbances of 1·5 or more, and assuming the fertilization to be a completely random phenomenon and the population of measured sperm heads to be representative of the total sperm population, the probability of the fertilizing spermatozoon having an absorbance of five or more will be highest for bull *C*, lower for bull *B* and lowest for bull *A*.

A fuller account of more extensive work is in progress and will be published elsewhere.

The samples of sperm were kindly supplied by the Scottish Milk Marketing Board's cattle breeding centres in Inchinnan and Perth.

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Giant Nuclei in the Earthworm *Lumbricus*

GIANT nuclei have been found by me in the epithelial cells of the pharynx in the earthworm *Lumbricus* sp. The pharyngeal epithelium of the earthworm was extensively investigated by Keilin[1], but no mention of giant nuclei was made in his paper.

Several hundred earthworms were purchased from a Kansas City supplier and were sectioned and stained with hæmatoxylin and eosin or by the Feulgen reaction. Giant nuclei were found most extensively in the pharynx and decreased in numbers toward the œsophageal region. Cytometric measurements were made using the greatest diameter and the diameter perpendicular to it. Fifty diameters of the more numerous small nuclei averaged 5·9µ. Fifty diameters of randomly selected giant nuclei averaged 21µ.

Giant nuclei were not found in *Lumbricus* sp. which were obtained from the following sources: a supply house in Chicago, Ill.; Chanahan, Ill.; Lyons, Kas.; Mission, Kas.; Oseola, Mo.; Weld, Maine; and other areas in Wisconsin, New York State and Canada.

Intranuclear morphology, such as chromocentres, was easily seen in these nuclei. In many instances the nucleolus was found to be bizarrely shaped. These nuclei appear to be polyploid because of the increased amount of Feulgen-positive material and an increase in the number of chromocentres present. Endopolyploidy is most highly expressed in gland cells and such an example is the pharyngeal gland cells of worker bees[2]. Painter[3] believes that there is a relationship between endopolyploidy and protein formation of these gland cells and that the increase of heterochromatic regions and nucleolar organizers is basic in this relationship.

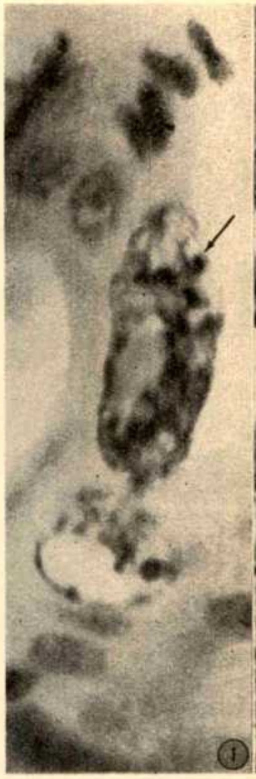

Fig. 1. Two giant nuclei showing chromocentres (arrow). Feulgen stain. (× *c.* 1,200)

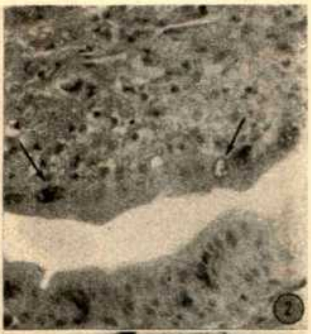

Fig. 2. Pharyngeal epithelium showing several giant nuclei (arrows). Hæmatoxylin and eosin stain. (× *c.* 37)

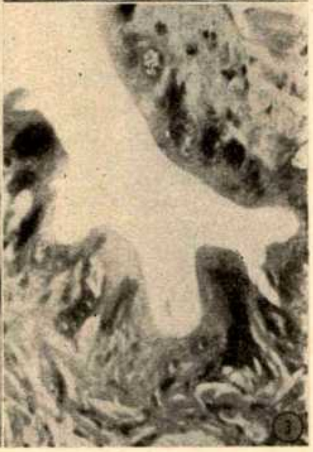

Fig. 3. Pharyngeal epithelium with many giant nuclei. Hæmatoxylin and eosin stain. (× *c.* 102)

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GENETICS

Linkage in *Coffea arabica*

THE *anormalis* factor (*Am Am*) of *Coffea arabica* affects the size of the plant, its branching habit and its leaves, flowers, fruits and seeds. The internodes of the branches are shorter than in the *typica* variety, taken as standard. The leaves are extremely abnormal, often almost palmate and of various sizes. The corolla has a variable number of lobes, the stamens are almost normal, but the style and stigma are very reduced and misshaped. The number of flowers and fruits is normal. The seeds have a slightly corrugated surface. A rather high percentage of locules contains more than one ovule. The *anormalis* shows incomplete dominance in F_1.

Table 1. NUMBER OF COFFEE SEEDLINGS OF THE VARIOUS CLASSES OBTAINED FROM CROSSES OF HETEROZYGOTES $\frac{Am\ An}{am\ an}$ WITH COFFEE PLANTS OF THE CONSTITUTION $\frac{am\ An}{am\ An}$

Hybrids	Total No. of coffee seedlings	No. of plants in the classes			
		$\frac{Am\ An}{am\ An}$	$\frac{am\ an}{am\ An}$	$\frac{Am\ an}{am\ An}$	$\frac{am\ An}{am\ An}$
H 3277 12-1-18-3-1 × *H* 1591-1	82	40	26	9	7
H 3287 12-1-18-6 × *H* 1591-1	52	19	23	6	4
H 3351 662 × *H* 1591-3	71	31	26	9	5
H 3352 662 × *H* 1591-4	23	7	9	2	5
H 3378 837 × *H* 1591-1	44	17	20	4	3
H 3379 837 × *H* 1591-3	25	14	5	3	3
H 3457 1184 × *H* 1591-1	51	24	21	1	5
H 3458 1185 × *H* 1591-1	116	53	49	1	13
H 3459 1215 × *H* 1591-1	40	27	11	0	2
H 3460 1216 × *H* 1591-1	112	50	49	0	13
H 3501 12-1-18-3-1 × *H* 1591-1	21	10	7	0	4
Total	637	292	246	35	64

The *anomala* mutant also affects the plant growth, its type of branching, the shape and size of the leaves, and the morphology of flowers, fruits and seeds. The number of flowers and fruits is very low and the occurrence of locules with more than one seed is, however, very high in some years. The *anomala* is recessive (*an an*), the F_1 being almost normal, with the exception that, occasionally, a pair of leaves develops with slightly misshaped apex.

The F_1 hybrids of *anormalis* and *anomala* are like *anormalis* heterozygotes; sometimes they have smaller and more irregular leaves, indicating a certain interaction of both factors. Back-crosses with *typica* or *bourbon* plants with the genotype *am am An An* gave indication (Table 1) of linkage of the two factors involved, with a recombination value of about 15 per cent. Due to difficulties in the separation of the classes $\frac{Am\ An}{am\ An}$ from $\frac{Am\ an}{am\ An}$, this recombination value is not precise and can be anywhere between 10 and 20 per cent. Back-crosses with the double-recessive type are difficult to obtain, due to the small number of flowers that it produces.

It is to be noted that the *anormalis* and *anomala* genes, producing rather similar phenotypes and having a very intensive pleiotropic effect, are, up to now, the only factors to give indication of linkage in *Coffea arabica*.

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Linkage between Deficiency of Glucose-6-phosphate Dehydrogenase and Colour-blindness

HEREDITARY susceptibility to hæmolysis by drugs and fava beans is believed to be due to a single sex-linked gene. This mode of inheritance would best explain the familial and population data, as summarized recently by Childs and Zinkham[1]. The main element of uncertainty regarding this hypothesis arises from the great variability of the measured blood abnormalities in heterozygous females (*in vitro* glutathione stability test and glucose-6-phosphate dehydrogenase activity).

However, sex-linkage of a trait may be established if it fails to show free assortment with a known *X*-linked character, like colour-blindness. Such a linkage study is being carried out in some of the Jewish communities in Israel which exhibit a high frequency of the enzyme deficiency[2].

Families characterized by a colour-blind son were investigated for enzyme activity. Only families in which both mutant characters segregated with their normal alleles were included in the investigation; each sibship exhibited only one type of colour blindness, according to the rough classification of the Ishihara tables.

Thirty-seven brothers of ten such families were found to segregate as shown in Table 1.

Table 1

	Number of brothers				
Colour vision:	Normal		Deficient		Total
Enzyme activity:	Normal	Deficient	Normal	Deficient	
Family No. 1		2	3		5
,, 2		2	1		3
,, 3		1	2		3
,, 4		2	1		3
,, 5		1	3		4
,, 6		2	1		3
,, 7		2	2		4
,, 8		1	1		2
,, 9		2	3		5
,, 10	1	—	—	4	5
Total	1	15	17	4	37

Each of these ten families thus comprises only two classes of sons, and there is complete lack of re-assortment between the two characters investigated. At the present stage, I am unable to account for the excess of repulsion between the two mutant genes.

These results indicate, therefore, a fairly close linkage between the loci determining colour-blindness and enzyme deficiency, and testify strongly against autosomal inheritance of the latter. They do not exclude partial sex-linkage of this trait, since families in which the trait would have been passed on from a father to all his sons in his *Y*-chromosome would have been excluded from the investigation because of lack of segregation.

I wish to thank Prof. Elisabeth Goldschmidt, of the Department of Zoology, Hebrew University, Jerusalem, for her advice and criticism, and Dr. Ch. Sheba, director of the Hospital and chief of the Department of Medicine (C), for his suggestions and encouragement.

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VIROLOGY

Relationship of Hepatoencephalomyelitis Virus and Reoviruses

In 1953, the isolation of a new virus termed hepatoencephalomyelitis virus was described[1]. This was recovered from the fæces of an aboriginal child suffering from a respiratory infection and who later developed alopecia areata. Further work[2] on this virus revealed the following properties: (1) Production of clinically recognizable disease only in suckling mice (commonly observed signs: oily hair, jaundice, peritoneal exudate, ataxia; a 'neurotropic' variant killed infant mice rapidly without these clinical signs). (2) Typical lesions in suckling mice were observed in the brain, cord, liver and heart. (3) Membranes of 110 mμ average pore diameter withheld virus; those of 130 mμ average pore diameter did not. (4) Resistance to ether. (5) Growth in tissue culture and chick embryos. (6) Infection in mice associated with the increased production of fæcal material of exceptionally high fat content. (7) The infection could be transmitted orally to suckling mice.

Agents which appear to be identical with hepatoencephalomyelitis virus have also been isolated in Holland by van Tongeren[3] from human fæces, and in India from mice by T. H. Work (personal communication, 1957).

In 1959, Sabin[4] proposed the group name 'reovirus' for a number of viruses distinct in size and other properties from other well-known enteric and respiratory tract viruses.

Subsequent investigations[5,6] have shown the ubiquitous nature of the reoviruses and revealed the following properties: (1) Membranes of 110 mμ average pore diameter withheld virus; those of 130 mμ average pore diameter did not. (2) Resistance to ether. (3) Growth in tissue culture. (4) Some strains grew in chick embryos. (5) Infection of suckling mice with lesions in the brain, heart and liver. (6) Production of a hæmagglutinin for human group 0 erythrocytes. (7) Three serological types have been determined.

Reoviruses with properties described above have been isolated from fæces of healthy children, children with diarrhœa and children with steatorrhœic enteritis; and from chimpanzees with rhinitis, a monkey with pneumonia, uninoculated monkey kidney tissue cultures, guinea pigs, mice and cattle[6,7].

The similarity of the hepatoencephalomyelitis virus and the reoviruses stimulated the further inquiry reported here. Through the courtesy of Drs. A. B. Sabin and Leon Rosen, the following strains of reoviruses, and antisera to them, were made available: type 1 ('Lang'); type 2 ('Amy' and 'Jones'); type 3 ('Dearing' and 'Abney'). Infection of suckling mice by the intraperitoneal route showed that both the 'Dearing' and 'Abney' strains of reovirus type 3 produced classical hepatoencephalomyelitis virus infection in suckling mice, with peritoneal exudate, oily hair effect, fæces with high fat content and lesions in brain, cord, heart and liver. Neutralization tests in suckling mice with 100 $I.D._{50}$ of hepatoencephalomyelitis virus gave the following results with hyperimmune sera against:

Type 1 ('Lang')	no protection at 1/10
Type 2 ('Amy')	no protection at 1/10
Type 3 ('Dearing')	complete protection at 1/160
Hepatoencephalomyelitis virus	complete protection at 1/320

Neutralization tests in suckling mice with 100 $I.D._{50}$ of reovirus type 3 ('Abney') gave the following results with hyperimmune sera against:

Hepatoencephalomyelitis virus	complete protection at 1/320
Type 3 ('Dearing')	complete protection at 1/160

In addition, (1) a common complement-fixing antigen was shown for both hepatoencephalomyelitis virus and the reoviruses; (2) hepatoencephalomyelitis virus produced a hæmagglutinin for human group 0 erythrocytes; (3) hæmagglutination-inhibition tests confirmed the neutralization tests; and (4) the type 3 reovirus ('Abney' strain) infects suckling mice by the oral route and produces the oily hair effect, and lesions in the liver, brain and heart.

These observations show that hepatoencephalomyelitis virus and the reovirus group are closely related, and that no differences are demonstrable with these tests between reovirus type 3 and hepatoencephalomyelitis virus.

The passage of hepatoencephalomyelitis virus in tissue culture or chick embryos aids in the selection of the 'neurotropic' variant, which does not produce the oily hair effect[2]. A similar change may have occurred in some strains of reovirus primarily recovered by tissue culture techniques. As all the reoviruses so far reported have been isolated primarily in tissue culture, and as the hepatoencephalomyelitis virus strains isolated have first been demonstrated in suckling mice (being particularly noticed by the oily hair effect), it is suggested that selection of variants could well occur in either host by continuous passage before testing the ability of the strain to infect a fresh host.

The facts that hepatoencephalomyelitis virus produces a disease of steatorrhœic type in mice and that the 'Amy' strain of reovirus was isolated from humans with steatorrhœic enteritis suggest that further inquiry should be made to assess the significance of these agents in the etiology of some forms of steatorrhœa in man.

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Use of Snake Venom Phosphodiesterase in Place of Pancreatic Ribonuclease for the Testing of Nucleic Acid Preparations from Tobacco Mosaic Virus

Deproteinized plant virus preparations are customarily incubated with pancreatic ribonuclease in order to show that their infectivity is not due to residual virus particles. With tobacco mosaic virus nucleic acid, exceedingly small concentrations of the enzyme (10^{-8} mgm./ml.) are sufficient to abolish infectivity after incubation for 24 hr. at room temperature, whereas tobacco mosaic virus is not affected

Table 1. Infectivities of Tobacco Mosaic Virus (TMV) and of Tobacco Mosaic Virus Nucleic Acid (TMV-NA) after Incubation with Venom Phosphodiesterase for 1 hr. at Room Temperature

Preparation	Concentration of diesterase (μgm. protein/ml.)	Infectivity of original preparation*	Infectivity in percentage of original			Lesion host
			Control†	Diesterase added		
				Prior to incubation	Prior to inoculation	
0·025 *M* glycylglycine buffer, *p*H 8·5 :						
TMV	280	20·7	84	91	111	*Datura stramonium*
TMV-NA	280	20·7	27	0	5	*Datura stramonium*
TMV-NA	3	9·0	2	0	5	*Datura stramonium*
0·025 *M* phosphate buffer, *p*H 7·5 :						
TMV	280	2·2	95	50	64	*Datura stramonium*
TMV-NA	280	48·0	133	0	26	*Datura stramonium*
TMV	56	4·9	198	47	45	*Datura stramonium*
TMV-NA	56	9·4	96	0	21	*Datura stramonium*
TMV	56	0·6	117	67	50	*Nicotiana glutinosa*
TMV-NA	56	15·7	42	0	39	*Nicotiana glutinosa*
TMV-NA	9	73·8	112	86	100	*Datura stramonium*
0·025 *M* acetate buffer, *p*H 5·1 :						
TMV	56	60·1	84	56	64	*Datura stramonium*
TMV-NA	56	76·4	62	46	114	*Datura stramonium*

* Average number of local lesions per half-leaf.

† Incubated at room temperature for 1 hr. in the absence of phosphodiesterase.

by a thousandfold higher concentration[1]. However, under these conditions tobacco mosaic virus nucleic acid incubated without enzyme often loses most or all of its activity[1]. Shorter times of incubation necessitate the use of higher ribonuclease concentrations, but at higher concentrations pancreatic ribonuclease, when mixed with plant viruses, inhibits infection[2-4]. This effect occurs immediately after ribonuclease is mixed with the virus and does not increase upon incubation of the mixture[2]. Consequently, as the ribonuclease concentration is increased and the incubation period shortened, the range within which tobacco mosaic virus nucleic acid is inactivated and tobacco mosaic virus is not affected becomes progressively narrower. If one considers that the effect on the virus of a given concentration of ribonuclease is furthermore a function of salt concentration and of concentration of tobacco mosaic virus[4], it becomes evident that the results of incubation studies with ribonuclease must be carefully evaluated before definite conclusions can be drawn.

An enzyme with more preferential action on virus nucleic acid and better stability in solution would be of value. Data presented here show that snake venom phosphodiesterase fulfils these requirements.

The phosphodiesterase was purified from eastern diamond-back rattlesnake venom by the method of Sinsheimer and Koerner[5]. The final preparation contained 2·8 mgm. protein per ml. It was stable at 4° C. for several weeks. Tobacco mosaic virus nucleic acid was prepared by the method of Gierer and Schramm[6]. Mixtures containing 0·5 ml. of an appropriate dilution of tobacco mosaic virus or tobacco mosaic virus nucleic acid, 0·5 ml. of 0·1 *M* buffer, and 1·0 ml. of diluted phosphodiesterase were incubated for 1 hr. at room temperature. Mixtures in which water was substituted for the enzyme or the enzyme was added prior to inoculation served as controls. Infectivity was assayed on plants of *Datura stramonium* L. or *Nicotiana glutinosa* L. by inoculation of 8–16 half-leaves with each preparation. Table 1 gives the results of some of the tests made.

Incubation with the phosphodiesterase led to complete inactivation of tobacco mosaic virus nucleic acid except in those cases where either the enzyme concentration was insufficient or the *p*H value was too low. Tobacco mosaic virus preparations retained approximately 50 per cent of their infectivity regardless of whether the virus was or was not incubated for 1 hr. with the enzyme. Virus preparations of very low titre still produced lesions after being incubated with high concentrations of the enzyme.

Thus, venom phosphodiesterase, like pancreatic ribonuclease, readily destroys the activity of preparations of tobacco mosaic virus nucleic acid. Unlike pancreatic ribonuclease, phosphodiesterase only weakly inhibits infection with the virus.

The preferential action of the phosphodiesterase may be a consequence of its high *p*H optimum. At *p*H 5·1, the enzyme had only a slight effect on the infectivity of tobacco mosaic virus nucleic acid. The virus, on the other hand, was equally inhibited at *p*H 5·1 and 7·5 whether it was incubated for 1 hr. or not. This suggests that the inhibition of tobacco mosaic virus infectivity was non-enzymatic or, if enzymatic, that it must have occurred during a sensitive stage after inoculation. Assuming that enzymatic action did occur after inoculation, the relatively slight effect of phosphodiesterase on virus infectivity can readily be explained by the unfavourable *p*H value for enzymatic action within the host plant.

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SOIL SCIENCE

C Horizon of the Soil Profile

A recent communication by Willimott and Shirlaw[1] refers to the *C* horizon and its absence in soils developed from fluvio-glacial and alluvial deposits near Durham City. These authors use a definition of *C* horizon as "unconsolidated parent material substantially similar to that from which the solum has developed". This is the definition of the U.S. Department of Agriculture[2], in which the *C* horizon is described as "weathered parent material" and *D* includes "any stratum which is not parent material". It is widely held that such a distinction between unconsolidated and consolidated parent material is difficult to maintain. In the case of a rock and an overlying drift or weathered material derived from similar rock, it has been the practice to describe the latter as parent material and the former as parent rock. Such a differentiation is not universally favoured, but an indexing of these cases as C_1 (unconsolidated parent material) and C_2 (rock, the weathering of which is producing C_1) is preferable to the use of D in this case. D as a symbol is then confined to material not contributing to the overlying solum but influencing it in some way, such as in its moisture relations. This use of C_1 and C_2 is that adopted by Kubiena[3].

It is, in practice, a difficult line to draw between unconsolidated and consolidated material, if the indexing of these is to separate them sharply as C and D. As an example of the difficulty if C is maintained only for unconsolidated material, we can consider a progression in parent rock from alluvial clay, through unconsolidated clay such as many Jurassic and more recent rocks, soft shales and harder Palæozoic shales, to Cambrian slates. At which point in such a series do we decide that the consolidation has proceeded so far that classification as C horizon is no longer appropriate ? The use of C_2 and C_1 as symbols indicates much more clearly the relation of rock and weathered rock to soil. It frequently happens with sedentary or nearly sedentary soils that rock weathering and soil formation take place so that A or A and B horizons overlie solid rock directly ; that is to say, pedogenic processes keep pace with rock weathering and no weathered rock exists which has not been subjected to further pedogenic weathering. All such soils on the U.S. Department of Agriculture system would be indexed as variants of A, D or A, B, D profiles. This seems inadequate to express relationships of soil and rock clearly. On the Kubiena system, the soils under discussion are classified as A, C_2 or A, B, C_2. A, B, D soils are confined to those in which the solum has no derivation, direct or indirect, from the D horizon.

Willimott and Shirlaw suggest that the absence of C horizons in soil profiles has not previously been reported for soils of the United Kingdom. It is, however, the general case that in layered alluvial deposits, material is often absent which can be accurately indexed as C horizon. In the common case of a medium or heavy-textured soil overlying gravel, the gravel is D horizon material, and profiles may vary rapidly in type between A, B, D ; A, B, CD, D or A, D, as is the case in alluvial soils along some rivers in North Denbighshire, surveyed with J. F. Harrop[4]. Such alluvial soils have been described and indexed as variants of A, B, D profiles in earlier *Memoirs of the Soil Survey of Great Britain*. For example, Muir[5], considering alluvial soils derived from varying proportions of Silurian and Old Red Sandstone rocks, indexes these as S, B, D or S, BC, D (using S to indicate the surface horizon of a cultivated soil, equivalent to A or Ap in some usages). Non-alluvial soils without C horizons are also described in the area covered by this memoir. Hobkirk and Faw series occur developed on thin till of Old Red Sandstone origin overlying Old Red Sandstone rock. This latter can vary greatly in lithology and is classified as D horizon. Muir states (*op. cit.*, p. 64) : "With this series (Hobkirk) it is unusual to find a C horizon, for the till is generally very shallow. The properties of the D horizon can vary greatly". Again, Glentworth[6], in his consideration of alluvial soils, indexed only an S horizon, while the Bogtown association, developed on what is believed to be interglacial lacustrine clay, is represented by one profile of horizons S and G only, which overlies a buried soil. Such composite profiles, in which C horizon material is no longer present, are frequent, especially in hill areas. Colluvial deposits may cause material derived from one rock-type to overlie another, also giving an A, B, D profile. In this case the C horizon material may be located higher up the slope, not in physical connexion with a solum developed from it.

In the final example quoted by Willimott and Shirlaw, it would appear helpful to consider using CD (or C_2D) as a horizon symbol. This indicates that the material so indexed has contributed in part to the overlying solum. The solum has, however, been partly derived from other sources. The CD definition indicates that such material also fills in part the definition of D material as a material influencing the solum, but unlike that from which the solum has been formed. The case of shallow soils developed from mixed till including limestone, overlying limestone, would then be indexed by variants of A, CD.

Such cases are widely met with in Britain ; for example, in my experience in both North and South Wales, where shallow drift overlies rock which is D or CD horizon. In Glamorgan, drift of gravelly outwash of mixed origin, and silty loam possibly derived from Carboniferous shales, both overlie Carboniferous Limestone, and where the drift is shallow, C horizon material may be missing. In North Wales, an A, CD profile is frequent in shallow soils, again over Carboniferous Limestone[4].

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D. F. **Ball** has raised a number of points arising out of our original communication on this subject. The distinction between the definition of soil horizons adopted by the U.S. Department of Agriculture[1] and that by Kubiena[2] turns on the question of the nomenclature of the solid rock. In the first system this would be designated as a D horizon and, in the other, either as a C_2 or a D horizon depending on its relationship to the regolith. Whichever system is adopted, and each has its merits, the essential requirement remains that a C horizon should bear a direct relationship to the solum developed. In a recent text it is noteworthy that Thompson[3] states that : "The recent trend is to describe the consolidated parent material below the C horizon (or immediately beneath the B horizon) as the D_r horizon".

It may therefore be useful to consider the advantages of the system accepted by the U.S. Department of Agriculture :

(1) This system has long been accepted by pedologists and any change, which may well cause con-

fusion, can only be justified provided it leads to clarity and facility in the recognition of debatable soil-horizons.

(2) The underlying unweathered solid rock cannot be accepted as part of the regolith ; to designate it as C_2 is therefore unacceptable.

(3) The correctness of the view that there is no necessary correlation between the "weathered parent material" and "unconsolidated parent material" is agreed. The *C* horizon of the U.S. Department of Agriculture may or may not be partly consolidated.

(4) We are dealing with soil formation, and the question whether the parent material is consolidated or not has no bearing on profile designation. There would seem to be no complication on the progression of parent materials from alluvial clay to Cambrian slates, cited by Ball, which incidentally can be found without resorting to widely different geological ages. On this interpretation, in the example quoted, the soft clay or shale, in the same way as the slate, is acceptable as a *D* horizon when it can be seen to be unweathered, that is, unaltered by pedogenic processes.

The findings of Muir[4] and of Glentworth[5] on certain of the soils of Scotland mentioned by Ball (*loc. cit.*), as well as the observations of Ball in Wales, are of interest in this context and we are indebted to him for directing our attention to them. These experiences offer substantial confirmation of the view that soils with no recognizable *C* horizon are widespread. Indeed, since the publication of our communication[6], profiles with similar characteristics have been encountered in aeolian soils in Libya[7]. A number of profiles, exhibiting a horizon succession of the *A*, *B*, *D* type, were studied in an area located about 25 miles south of Misurata in the region of Taourga oasis (Tripolitania). Soil profiles with a horizon succession of *A*, *D* type were also recorded. It is hoped to publish details of the Taourga soil survey at a later date. Furthermore, in the course of a detailed survey of Malta, Bowen-Jones and Dewdney[8] reported very shallow loams developed over limestone in which a succession of *A*, *D* horizons was recognized in the profile. Here admittedly human activity had contributed in part to the soil material ; nevertheless, on level areas little exposed to soil wash, an *A*, *D* profile develops with time. Thompson (*loc. cit.*) also reports profiles of this type in the United States.

The designation of a *CD* horizon in the profiles developed over magnesian limestone in County Durham may provide a solution to the difficulty previously discussed[6]. On the basis of the U.S. Department of Agriculture classification of a *D* horizon, the *CD* indexing would appear to be valid since it indicates the dual role taken by this horizon in supplying part of the parent material and as constituting the underlying rock. A clear definition is necessary, however, since other profiles may carry a similar designation but with different significance. For example, certain rendzinas[9] are indexed *A*, *AC*, *C* ; in this case the significance of the *AC* designation is that the horizon represents a transitional zone only between *A* and *C*. Again, the designation *AG* indicates that the *A* horizon is gleyed.

Soils in which no true *C* horizon can be observed represent a special case. It may be useful to consider three typical examples, namely : alluvial soils, shallow drift soils and deep fluvioglacial soils.

Alluvial soils. Some of the soils developed on alluvium referred to by Ball, for example, the Bogtown association studied by Glentworth[5], exhibit a solum which is still actively developing and evolving. Here the solum is shallow and may actually be accumulating. The true parent material is all transported. Furthermore, the pedogenic process is keeping pace with the deposition of parent material. In this case the absence of a true *C* horizon is inevitable so long as the deposit is shallow. Although the alluvial deposits studied at Durham[10] were much deeper (up to 10 ft.), a true *C* horizon was again absent.

Shallow drift soils. In the shallow drift soils of the Hobkirk and Faw series, studied by Muir (*loc. cit.*), the parent material was originally derived from a distant origin. This forms drift, which after pedogenesis is too shallow to retain an unaltered layer of parent material below the solum. Here the *C* horizon occurs only in the initial stages of solum formation.

Fluvioglacial soils. In the deep fluvioglacial soils observed at Durham[11] the potential parent material, up to 23 ft. thick, is composed of thin beds of heterogeneous material—gravel, sand and clay. With the development of *B* horizons, solum formation occurs down to a depth varying roughly between 6 ft. and a maximum of 12½ ft., and is underlain basically by *D* horizons. Owing to the varied and heterogeneous character of the deposits, a *C* horizon is not now present. In the course of time, pedogenesis allows successive *D* horizons to be transformed into true *B* horizons. Whichever method of classification is applied, the successive horizons beneath the *B* horizons would be designated *D*.

It is therefore inevitable that in the case of the shallow alluvial and the shallow drift soils, profiles of the *A*, *B*, *D* type will develop. In both cases these represent very shallow soils. The fluvioglacial soils are different because there is adequate parent material which has been exposed for a period sufficient for a complete soil profile to evolve. Here the *A*, *B*, *D* type soils are the result of the inherent geological properties of the fluvioglacial deposits which, in time, allow a succession of *D* horizons to change into *B* horizons as the profile evolves. It was to this latter class of *A*, *B*, *D* type profiles that we wished originally to direct attention.

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Phosphate Requirement of Lupins grown as a Pioneer Crop on Culm Measure Soil with Impeded Drainage

Lupins are reputed to be one of the most efficient extractors of phosphate from soil among farm crops and have been used extensively on the Continent as a pioneer crop on soils of very low phosphate and lime status.

In order to explore the possibility of making use of lupins as a pioneer crop on Culm Measure soil which was poorly drained, acid and of low phosphate status, a newly cleared field at the North Wyke Experimental Station, Devon, was sown with lupins in 1958. In this field an observational experiment with phosphate was conducted in which a large response to superphosphate was observed. Consequently, in 1959, a replicated field experiment was laid down in order to determine if this effect was due to phosphate or to other constituents of superphosphate.

The results of this experiment are given in Table 1, from which it can be seen that the very marked yield responses are all associated with phosphate applications, indicating that the effect of superphosphate was due to its phosphate and not to any other constituent.

Further, the figures for phosphorus content in the plant dry matter strongly suggest that the yield responses were caused by phosphate applications and not, for example, by a phosphate/molybdenum interaction[1]. The similarity between the plant nitrogen contents also suggests that the supply of trace elements was adequate.

This experiment does not support the assumption of long standing that lupins are able to obtain phosphorus from relatively insoluble soil phosphates. This finding is in agreement with an observation made by Schofield and Nethsinghe[2], who equilibrated a mixture of a surface soil derived from Upper Greensand and river sand with phosphate labelled with phosphorus-32 and grew cabbage, barley and lupins in the mixture. Allowing for phosphorus in the seeds, they found that six weeks after potting, the specific activity of the phosphate taken into the plants was the same for all three species, and was equal both to that in the solution after equilibration and to that in the calcium chloride extract of soil from similarly treated pots without plants. Hence none of the crops, including lupins, mobilized insoluble phosphates, that is, isotopically non-dilutable phosphorus.

When lupins can thrive without phosphate application, even on soils with a very low phosphate status, the explanation is more likely to be an ability to obtain phosphate from the deeper layer of the subsoil outside the reach of more shallow rooting crops. In the experiment described, impeded drainage possibly prevented the lupins from drawing on phosphate reserves in the subsoil and consequently the crop was very responsive to phosphate.

Table 1. PHOSPHATE EXPERIMENT IN LUPINS (VARIETY WEIKO III) SOWN JUNE 5, HARVESTED AUGUST 29, 1959

Soil: Culm Measure, impeded drainage with clay subsoil. History: old woodland cleared in 1957 and fallowed in 1958. Soil analysis: citric-soluble phosphate, 50 p.p.m., and potassium (K_2O), 184 p.p.m.; organic carbon, 3·1 per cent; *p*H in water, 4·2

Treatments 1–4 received 80 lb K_2O/acre plus the following:	Rate of application per acre	Yield of dry matter (cwt./acre)	Phosphorus in dry matter (lb./acre)	Percentage in dry matter			
				P	K	Ca	N
1 Nil	—	2·2	0·25	0·105	1·63	0·203	2·81
2 Single superphosphate	120 lb. P_2O_5	17·1	4·03	0·206	1·73	0·352	2·53
3 Monocalcium phosphate	120 lb. P_2O_5	15·7	4·06	0·227	2·02	0·315	2·40
4 Gypsum	As present in 2	2·8	0·31	0·099	1·57	0·274	2·77
5 Monopotassium phosphate	120 lb. P_2O_5	19·9	4·66	0·211	1·89	0·283	2·48
LSD (0·05)		6·4	1·72	0·029	NS	0·019	NS
LSD (0·01)		9·3	2·50	0·041		0·027	
S.E. per plot		3·4	0·91	0·015	0·26	0·010	0·34

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PSYCHOLOGY

Measurement of Psychomotor Perseverative Tendencies

One of us (S. D. P.)[1] has described what he believes to be a novel procedure in psychological research. It is based on a well-attested observation that, if individuals repeat after a brief interval of time a section of a simple maze, some very surprising perseverative tendencies become apparent.

When shuffled among a group of similar maze threadings by other individuals, the first performance can be correctly matched with the second in about 90 per cent of cases, even when names or all other identifying marks are concealed from view. The percentages correctly matched have been tabulated for 800 pairs of performances, and show apparently little differences in the matching ability of experienced observers. The phenomenon is considered remarkable on two counts. In the first place it is surprising that recognizable patterns occur on the basis of a single repeated performance of an activity which is new in the experience of the subject. As is well known, habits of action are ordinarily established only on the basis of very many repetitions of the activity in question.

In the second place, the matching process depends on similarities in lines and turnings at eight different points. The performance is so arranged that it would be difficult, not to say impossible, for the individual to recall consciously the kinds of lines or the types of turns he made on his previous attempt. This unawareness is heightened by the fact that his attention is distracted by the necessity of choosing the course to be pursued to find his way out of the maze. Yet though he may be unable to describe verbally or to demonstrate the kind of turn he made at any particular point, yet he usually tends to repeat his original pattern. The strength of this tendency varies; in some individuals it is so strong that the pattern is still clear after a year's interval between performances; in 10 per cent the repetitive tendency is so weakly impressed that their records are unmatchable even when the lapse of time is only 20 min. It may well be that the distraction of planning the course allows subconscious retention to play its full part. There seems no reason for believing that this perseveration differs in nature from that displayed in gestures or other automatic expressive movements.

However, the demonstration of this phenomenon would amount to little more than a psychological stunt if the pattern of similarities cannot be expressed in the form of a numerical score that would represent the strength of the subconscious tendency. We and our co-workers at the University of Hawaii have devoted considerable time and effort to developing such a score by projecting the paired performances on a large screen. Four psychologists, having first set up standards of comparison, allotted points to each pair of turns in the maze performance according to their degree of similarity, and also to the place of beginning and to the style of going out the final exit. These ratings were then averaged and summated as a score. It seemed to be a safe conclusion that very high scores distinguished individuals with rigid standards of performance, whereas low scores signified less consistent or persistent methods of motor expression. No value judgments with regard to these differences can be assigned at the present stage of our research. The system of allotting conformity-flexibility scores was described and discussed in the articles cited. Obviously, definite theoretical assumptions were premature until more objective scoring methods could be used.

We are now exploring what promises to be a much more reliable approach. We are attempting to devise an IBM scanning procedure such that the pencil tracing on the Porteus Maze will in effect be plotted and recorded in an IBM 650 co-ordinate system. We propose to use in a first possible method of scanning an opaque, enlarged projection of a Porteus Maze section on a mark-sense IBM card. An experimenter will then fill in those circles on the mark-sense cards that the line tracing from the Porteus Maze crosses. From the mark-sense card the IBM machine will automatically punch a regular card.

An alternative method will discard the use of the opaque projector should it prove to be too unreliable for an experimenter to follow the enlarged tracing on an IBM card. With this method we will attempt to print enlarged photographs of the pencil tracing on sensitized IBM cards. The experimenter then might find it easier to fill in the mark-sense circles and his measuring discriminations therefore might be more reliable. When our scanning procedure is perfected, with proper subsequent programming, we should be able to score in a completely objective manner characteristics of the pencil tracings that have previously been found to be important in the matching process.

Once a reliable measure of the perseverative tendency has been achieved, we can then study the behaviour of groups segregated by age, sex, race, intelligence, social adjustment, mental stability, etc. Whether or not the procedure will demonstrate the influence of subconscious retention is not at present of crucial importance.

The fact that the new approach differs from other personality assessments is worth emphasizing. It does not depend on questionnaires, or verbal reports from the subject. He is not required to reproduce or imitate, so that no creative or artistic ability is involved. The situation exhibits a minimum of external structuring, but on the other hand presents to the subject of the experiment a maximum of opportunity to display his characteristic styles of response, with no intervention by the observer. Nor does the former have any knowledge of the significance of his reactions. Hence the scoring comes closer to what Eddington has termed pointer readings, so familiar to physicists and chemists. It is true that experimental psychologists also use similar measurements in studying sensory phenomena. We believe that our approach takes in larger sections of behaviour than if the eye or the ear is being studied, and is less likely to issue in a psychology of 'bits and pieces'. In other words more of the whole man is engaged and the style of his habitual or everyday reactions is more definitely indicated.

It is perhaps unfortunate that the Porteus Maze Test, first published forty-five years ago[2], forms the basis of the new procedure, since psychologists may easily jump to the erroneous conclusion that this constitutes merely another application of a very old test. As there are neither right nor wrong responses, and hence no successes or failures as in the Maze Test, the two applications are entirely different. The situation is analogous to the two uses of a hammer, which can be used for driving in or extracting nails, dependent upon which end of the hammer-head is employed. The main connexion with the test is that eight designs of a regular Maze series are first threaded by the subject before he is asked to repeat the eleven-year design which occurs about the middle of the series. The sole aim of this is to interpose a number of other stimuli and responses so as to make it unlikely that the individual will have any conscious recollections of the details of execution previously carried out. The essential difference is that the psychologist is now interested in what the individual does, and not in what he ought to have done.

The main reason for this communication is the realization that the task of developing more exact methods and their application is difficult. It is hoped that appreciation of the new avenues of potential psychological inquiry which could be opened up may attract the interest and co-operation of others. As one example, it would be indeed interesting to discover at what age we tend to become 'set' in our ways and thus less flexible in action or judgment, or whether some individuals are immune from this process of 'neurosclerosis' as an accompaniment of ageing.

A secondary aim is to make the methods used more intelligible to scientists than are some of the clinical approaches of psychology and psychiatry. The fact that funds may not be available here for the continuation of the project makes it all the more desirable that other workers be more fully appraised of an approach that appears to be promising for the further study of human behaviour.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, February 27

Operational Research Society (in the Lecture Theatre of the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Ir. A. H. Schaafsma (Holland): "The Meaning of OR for Production Planning".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Sir John Hunt, C.B.E.: "A Summer Expedition to Greenland".

Tuesday, February 28

Royal Institution of Naval Architects (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Mr. J. M. Murray and Mr. H. N. Pemberton: "Safety of Nuclear Marine Propulsion Plant".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. G. Spencer: "Hypertension and Unilateral Renal Disease; the Selection of Patients for Surgery".* (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

University of London (at the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. A. B. Hart: "The Fuel Cell".* (Second of five lectures on "Generation of Electricity without the use of Rotating Machinery".)

Illuminating Engineering Society (at the Royal Institution, Albemarle Street, London, W.1), at 6 p.m.—Sir Graham Sutton, F.R.S.: "Long Range Weather Forecasting". (Sixth Trotter-Paterson Memorial Lecture.)

Institution of Mechanical Engineers, Steam Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Advantages of Increased Pressures and Temperatures and Ratings of Large Steam Power Plant".

Society for Analytical Chemistry, Physical Methods Group (in the Cuthbert Wallace Lecture Room, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2), at 6 p.m.—Meeting on "Physical Methods of Analysis Used in Medical Research".

Society of Chemical Industry, Corrosion Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Cathodic Protection Panel Meeting. Mr. M. G. Duff and Mr. P. Clark: "External Protection of Floating Craft".

Society of Instrument Technology (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Dr. V. S. Griffiths: "Three Terminal Bridge Measurements and Automation".

Wednesday, March 1

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. D. G. King-Hele: "Satellites and the Earth's Outer Atmosphere" (Symons Memorial Lecture).

British Institution of Radio Engineers, Television Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. L. H. Bedford: Inaugural Address.

Institution of Electrical Engineers; and the Royal Aeronautical Society (at the Royal Aeronautical Society, 4 Hamilton Place, London, W.1), at 6 p.m.—Discussion on "The Training of Electrical and Electronic Engineers for the Aircraft Industry", opened by Mr. G. S. Bosworth.

Institute of Physics and the Physical Society, X-ray Analysis Group (at 47 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. W. A. Wooster: "Crystal Symmetry—Old and New".

Wednesday, March 1—Thursday, March 2

Iron and Steel Institute, Iron and Steel Engineers Group (at 4 Grosvenor Gardens, London, S.W.1) at 2.30 p.m. on Wednesday and 10 a.m. on Thursday—Forty-second Meeting.

Thursday, March 2

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. J. C. Ruegg: "On the Tropomyosin–paramyosin System in Relation to the Viscous Tone of Lamellibranch 'catch' Muscle"; Miss Jean Hanson and Mr. J. Lowy: "The Structure of the Muscle Fibres in the Translucent Part of the Adductor of an Oyster".

Institute of Physics and the Physical Society, Acoustics Group (in the Physics Department, Imperial College of Science and Technology, South Kensington, London, S.W.7), at 5.30 p.m.—Mr. A. E. Crawford: "Lead Zirconate–Titanate Piezoelectric Ceramics".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. N. N. Hancock and Mr. P. L. Taylor: "The Place of Formal Study in the Post-Graduate Training of an Electrical Engineer".

Royal Institution, Library Circle (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Mr. A. K. Parker: "The Function of a University Press".

University of London (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. D. N. Nabarro: "Oral Hypoglycaemic Agents".* (Last of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Institute of Metals, London Local Section (at 17 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. J. A. Catterall: "Progress in the Electron Theory of Metals".

Royal Institute of Chemistry, London Section (at West Ham College of Technology, Romford Road, London, E.15), at 7 p.m.—Dr. S. A. Miller: "Chemicals from Acetylene".

Friday, March 3

Linnean Society of London (at Burlington House, Piccadilly, London, W.1), at 10.45 a.m.—Symposium on "The Experimental Taxonomy of Flowering Plants". Speakers include Prof. D. H. Valentine, Dr. P. H. Raven, Dr. A. D. Bradshaw, Dr. N. W. Simmonds, Dr. J. R. Ellis, Dr. M. Borrill, Dr. K. Jones and Prof. I. Manton.

Society for Analytical Chemistry (in the Meeting Room of the Royal Society, Burlington House, Piccadilly, London, W.1), at 2.15 p.m.—Annual General Meeting, followed by Mr. R. C. Chirnside: "The Enlargement of Horizons in Analytical Chemistry".

Association of Consulting Scientists, and the Forensic Science Society (at the School of Pharmacy, Brunswick Square, London, W.C.1), at 5 p.m.—Colloquium on "The Expert Witness".

Institution of Electrical Engineers, Medical Electronics Discussion Group (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "The Clinical Value of E.E.G. Recording" and "Present Trends in General Purpose E.E.G. Recorders", opened by Mr. H. B. Morton and Mr. H. R. A. Townsend.

Royal Aeronautical Society, Rotocraft Section (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. H. W. Mitchell and Mr. S. G. Lennox: "Helicopter Approach Aids".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Sir Lawrence Bragg, F.R.S.: "The Development of X-ray Analysis".

Saturday, March 4

London County Council (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. A. W. Smith: "Explorations on the Bolivia–Peru Border".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned:

Assistant Experimental Officer (with at least G.C.E. in five subjects including two (science or mathematics) at "A" level, or equivalent, and laboratory experience in chemistry or biochemistry) to assist with investigations on sheep metabolism—The Secretary, Institute of Animal Physiology, Babraham, Cambridge (March 4).

Assistant in Biochemistry in St. Salvator's College—Joint Clerk to the University Court, College Gate, St. Andrews (March 4).

Head (highly qualified experienced person) of the Department of Physics—The Bursar, College of Advanced Technology, Gosta Green, Birmingham 4 (March 5).

Scientific Officer (with a good honours degree in mathematics) or Assistant Experimental Officer (with a pass degree or equivalent) in the Statistics Section, to do advisory work on the design and analysis of experiments, and research work on biometrical problems—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (March 6).

Leverhulme Research Fellows (2) (suitably qualified graduates) in Radio-Astronomy, for research in some aspects of radio-astronomy, using the radio-telescope at the University's Nuffield Radio-Astronomy Laboratories at Jodrell Bank—The Registrar, The University, Manchester 13 (March 7).

Lecturer in Physics—The Registrar and Secretary, King's College (University of Durham), Newcastle upon Tyne 1 (March 10).

Senior Lecturer in Mathematics at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, March 10).

Deputy Librarian (with a good honours degree and experience in a university or similar academic library, and preferably with professional library qualifications)—The Secretary, University College, Gower Street, London, W.C.1 (March 11).

Leverhulme Research Fellow in the History and Philosophy of Science—The Registrar, The University, Leeds 2 (March 17).

Assistant in Biochemistry in the Department of Physiology and Biochemistry, Queen's College—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (March 18).

Assistant Lecturer (with a special interest in experimental psychology) in the Department of Psychology—The Secretary, University College, Gower Street, London, W.C.1 (March 18).

Lecturer in Geography—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (March 18).

Senior Lecturer or Lecturer (graduate in medicine, with special experience in virology) in the Department of Bacteriology—The Secretary, The University, Aberdeen (March 18).

Lecturer or Assistant Lecturer (preferably specialized in inorganic chemistry) in Chemistry at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 20).

Lecturer in Physics—The Secretary, The University, 38 North Bailey, Durham (March 25).

Senior Lecturer and a Lecturer in the Department of Physiology, University College, Ibadan, Nigeria—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (March 27).

Biochemist (with postgraduate experience and preferably some knowledge of bacteriological techniques) to assist in a research project on bacterial polysaccharides—The Director, Central Enteric Reference Laboratory, Central Public Health Laboratory, Colindale, London, N.W.9.

Editorial Assistant (with a university degree in science or technology, and preferably at least two years postgraduate experience in science or industry)—The Manager, *Nature* Office, Macmillan and Co., Ltd., St. Martin's Street, London, W.C.2.

Experimental Officer (graduate, or with A.R.I.C., H.N.C., or an equivalent qualification and experience in the use of techniques of paper and column chromatography) in the Nutrition Department, for biochemical research on problems of protein and amino-acid metabolism—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/6.

Fellow or Lecturer in Philosophy—The College Secretary, Balliol College, Oxford.

Head (with the qualifications, teaching and research experience necessary to secure recognition as a teacher of the University of London) of the Department of Physics—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

Laboratory Technician (with biochemical experience) to work in the Department of Chemical Pathology on research connected with muscle diseases—The Secretary, Institute of Neurology, National Hospital, Queen Square, London, W.C.1.

Masters to teach Physics; Mathematics; and Geography—The Headmaster, King's College School, Wimbledon, London, S.W.19.

Senior Lecturer (physicist, with experience in university teaching and interested in the problems of radiation safety) in Health Physics, for duties which will include the organization of postgraduate courses on the nature and control of radiation hazards, inauguration of research and the supervision of radiation safety throughout the College—The Secretary, Royal College of Science and Technology, George Street, Glasgow, C.1.

Senior Lecturer (with qualifications and experience to teach mathematics for the Diploma in Technology courses and for Degree courses in engineering and science) in Mathematics—The Registrar (Dept. D), Bradford Institute of Technology, Bradford 7.

Senior Physicist (with some experience in hospital physics) to act as deputy to the Principal Physicist and to take responsibility in radiation physics and isotope work—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Medicine, Science and the Law, Vol. 1, No. 1 (October 1960). Edited by Francis E. Camps. (Official journal of the British Academy of Forensic Sciences.) Pp. 132+viii. Published quarterly in October, January, April and July. Annual subscription 63*s*. (U.S.A. 10 dollars.) Single issues 17*s*. 6*d*. (London: Sweet and Maxwell Ltd., 1960.) [2111

United Kingdom Atomic Energy Authority: Research Group. Report, AERE-R 3349: The Deposition of Fission Products from Distant Nuclear Test Explosions—Results to the Middle of 1960. By R. N. Crooks, Miss E. M. R. Fisher, M. J. Owers and T. W. Evett. Pp. 12. (Harwell: Health Physics Division, A.E.R.E., 1960. Available from H.M. Stationery Office, 1960.) 2*s*. 6*d*. net. [2211

Harper Adams Agricultural College, Newport, Shropshire. Technical Bulletin No. 3: Maize as a Silage Crop. By M. Eddowes. Pp. 13. 3*s*. 6*d*. Technical Bulletin No. 4: The Control of Weeds in Maize. By M. Eddowes. Pp. 14. 3*s*. 6*d*. (Newport: Harper Adams Agricultural College, 1960.) [2211

Welcome, Stranger. By Ronald Smurthwaite. Pp. 16. (London: British Association for Commercial and Industrial Education, 1960.) 2*s*. 6*d*. [2211

Ministry of Housing and Local Government, and Scottish Office. The Surface Water Year-Book of Great Britain, 1958–59: Hydrometric Statistics for British Rivers, together with related Rainfalls for the year ended 30th September, 1959. Pp. xiv+92. (London: H.M. Stationery Office, 1960.) 17*s*. 6*d*. net. [2211

Ministry of Agriculture, Fisheries and Food. Fishery Investigations. Series II, Vol. XXIII, No. 2: Hydrographical Observations in the Irish Sea, January–March 1958. Pp. ii+25. (London: H.M. Stationery Office, 1960.) 8*s*. 6*d*. net. [2411

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SCIENCE AND THE HUMANITIES

JUST over a century ago, J. S. Mill, in his famous essay on liberty, remarked that "the general tendency of things throughout the world is to render mediocrity the ascendant power among mankind". He added that there is always a need for persons not only to discover new truths and point out when what were once truths are true no longer but also to commence new practices, and set the example of more enlightened conduct, and better taste and sense in human life. All that is still profoundly true to-day, and in fact the advent of the mass media of communication has accentuated the danger of mediocrity or worse. Those passages could well have been in the mind of Dr. Caryl Haskins in writing his report for 1959–60 as president of the Carnegie Institution of Washington*, which once again gives a percipient and imaginative survey of the inter-relations of the scientific disciplines with themselves and with the society in which they are nurtured.

While the present report follows closely on last year's report and develops further the same lines of thought then advanced, particularly those arising out of consideration of a real or imagined dichotomy between the scientific and non-scientific world, such as Sir Charles Snow discussed in his Rede Lecture, "The Two Cultures", it is nevertheless fully independent. In her recent life of Lord Lugard, in writing of Lady Lugard, Dr. M. Perham observes: "Even to-day . . . the exceptional and independent mind can often be traced to some divergence from the usual course of schooling which has given both stimulus to thought and freedom to read at will". Although Dr. Haskins's address can scarcely be described as an exposition of this text, it is clear enough that he is profoundly disturbed at some educational tendencies to-day, and that he places very high among his presidential responsibilities that of fostering the exceptional and independent mind, and giving such minds every opportunity to render the contributions to scientific and technological advance which they alone can make.

Dr. Haskins begins by emphasizing the vistas of insight and wonder inconceivable to our forefathers, which we enjoy because of the unparalleled advances in our appreciation and comprehension of Nature, and suggesting that, in consequence, this generation may be standing at a major cross-road of history and living in that deep travail which more than once has preceded and ushered in a Golden Age. The brilliance and variety of man's concepts to-day, the diversity of his imagination, the extent of his practical power to shape and to use his material environment, perhaps even the penetration of his understanding, have never been remotely rivalled in all earlier history, nor have the dangers of his very existence seemed as vivid, as omnipresent, as remote from resolution. While we are convinced that the values in which our society is rooted are fundamental to us, yet we are constantly haunted by the feeling—and the evidence—that the means by which we have traditionally implemented and expressed them in the past, if projected into the coming years without innovation and imagination, will be inadequate to our future.

What is less obvious is the challenge that this contrast presents, and Dr. Haskins reminds us that the great ages of mankind, the ages of the most radical changes and the biggest advances, the ages that later generations of men called great, were not the times of easy optimism when man thought everything discovered, everything designed, everything finished. As Whitehead wrote, on the whole the great ages have been unstable ages. Greatness and ease, vast innovation and untroubled stability, are no more compatible among nations or in a world community than they are among individuals. To-day the United States is predominantly a scientific and technical nation, living in an age itself characterized by the explosive development of science throughout the world, and Dr. Haskins asks accordingly whether there may not be significant things which the very structure of science as a way of life could say in aid of the understanding of ourselves, of this age and the task of the American nation—of an age and a nation profoundly shaped by science at a secular level and at a deeper and more spiritual level too.

That question could well be addressed to Britain also, and the answer we give to it should determine very largely the answers to be given to many of the questions now arising in regard to the expansion of university and technological education, of specialization in the sixth forms of schools and elsewhere, and of the nature and extent of general education. Dr. Haskins develops his theme with imaginative insight to show how in the growth of modern science from the time of Leonardo da Vinci it has been sensitively responsive to the moods and needs of men, portraying accurately the successive ages which nourished it. Everywhere it took its character from the conspicuous needs and opportunities of the time, developing greatness in those areas where it was most needed; and in turn its spirit was everywhere reflected in the society that it served. Surely it is not accidental, he argues, that the centuries of the flowering of modern science have also been the centuries of the rise of the Western concept of the dignity, the uniqueness, and the essential worth of the individual, or that these were also the centuries of the genesis and ascendency of the modern Nation-State as we understand the term to-day.

While in these four centuries there have been comparatively static or satisfied interludes, the main trends in science have been marked by instability, vast shifts in man's concepts of his universe and his place in it, and of his own very constitution and nature, delight in ideas, or fascination with them because of their compelling sweep and force, and an overwhelming urge to communicate them broadly and to share widely in them. The ages that have witnessed these trends have been characterized, too, by the attainment of new levels of physical well-being and of the moral and spiritual horizons and opportunities of the individual. Simultaneously have come, both as cause and consequence of these developments, demands for innovation in the structure of society, reflected in social and political insecurity and in rapidly shifting patterns of political and social organization. The more we contemplate our own time, the more it seems to resemble those earlier dynamic and critical eras of Western history, typically characterized by a keen sense of change and exploration and also by an atmosphere of personal, political and social insecurity. The eagerness with which developments in the exploration of space are watched is only an illustration of the sense of new physical frontiers close at hand, the ferment of new ideas and the vast shifts in the individual's notion of the universe and his place in it, of his very nature, that mark our time like those of Leonardo, Columbus, Magellan and Vasco de Gama. There are the same new and violent and little-understood demands of political and social organization and the same intense need for vigorous and imaginative leadership in every field.

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* Carnegie Institution of Washington. The Report of the President, 1959–1960. Pp. xiii–lxxiv. (Reprinted from *Carnegie Institution of Washington Year Book*, 59.) (Washington, D.C.: Carnegie Institution of Washington, 1960.)

Dr. Haskins does not omit to point out that some of the changes with which we are concerned so vastly exceed those that occupied other ages as almost to differ in kind. The magnitude and variety of our sources of power, the capacity for essentially instantaneous communication have, for example, forced a major human adjustment on the West before it would accustom itself to this range and speed of communication, and new nations are called to make the same great orientation in a world which allows little time for major re-adjustments of international affairs. Further, new levels of human integration are demanded by the very pressure of population and the intricacy of modern life, and with the control, modulation and organization of the new sources of physical power calling for particular attention, an order of leadership and of excellence in every sphere greater than we have yet known is also demanded—a leadership of greater force, greater knowledge, greater understanding, and one more widely dispersed in every level of our society.

Here, in touching on science and leadership,' Dr. Haskins is concerned, not so much with the leadership of science itself, as in relation to the position of science within the framework of the character, the aspirations, and the contemporary needs of a nation, particularly the United States. He stresses the value placed on individual excellence, the dependence of the essential worth of life for the individual in a dedication larger than himself, and the way in which such total dedication spans the work-day world no less than the religious realms. Reasoning thus, the supreme importance which science attaches to the individual leads him to regard science as one of the spiritual factors in a democracy, closely resembling in its own society that of a democracy, deeply motivated by an ethic at once basically religious in character and dedicated both to the supremacy of the individual and to that spirit of frontier uncomformity which is a pronounced and precious element in the American tradition.

Science, then, is one of the most precious spiritual and moral, as well as material, values in our society, and it serves us to-day in a particularly critical time, and nowhere more so than in its emphasis on the individual. Originality and independence, individual dissent, properly protected, are the very essence of our civilization ; and for science in its public function, the protection of the individual and his independence, the honour of free thought and free expression and the public welcome of dissent are all vital. To-day, however, they are threatened, not so much by formal oppression or suppression, or organized intolerance, as by more subtle and insidious factors—inattention, lassitude, hostility to innovation through misunderstanding of its nature and effect, especially the threat of simple, widespread, massive misunderstanding of the nature of the scientific way, its purpose and its indispensable place and part in our practical world and in our deepest philosophy and belief.

At this point Dr. Haskins returns to the analysis of the apparent dichotomy of the two cultures on which he touched in his previous presidential report, and, like Sir Cyril Hinshelwood, he regards the common factors that unite all the creative disciplines, be they artistic or humanistic, philosophical or scientific, as far deeper and more fundamental than the more obvious differences that distinguish them. Pointing out that in the earlier days of scientific effort, before the sheer weight of learning and evidence forced our modern specialization, many great figures frequently united the two fields, he stresses the essential identity, at their most general level, of the characteristics and objectives of the arts and of the sciences. Both are primarily concerned with the process of discovery itself, and with discerning the beauties of Nature. Both professionally excel in innovation, and take delight in the shapes of things, their patterns, lights and shades, and in the uncovering of hitherto unperceived relations. Both seek to cast their descriptions and discoveries in evocative and universal forms, and at a very profound level of understanding ; both take communicability and coherence, within the subject itself and with the audience, as the ultimate criterion of conceptual or of demonstrated truth. Nor is this all. Both take their very living being and draw their vitality from the joy of discovery, from the attaining of ends unknown when the work was begun, through means which are indeed partly or wholly unknown, but usually of technical difficulty. Both are deeply concerned with the underlying likeness between superficially unlike things ; with the equating of different aspects of the same phenomena; with uniting such apparently disparate aspects in common matrices; with the generalizations which follow painstakingly minute analyses, with analogies and their testing. Typical of all the most creative and imaginative pursuits of man, of the life processes themselves, the arts and the humanities, quite as much as the natural sciences, must be truly consonant with our contemporary history; and as faithfully as they reflect their period, so they are reinforced by the societies that nourish them.

There are, of course, important differences between the disciplines of the arts and the humanities existing at another level, and these too must be fully recognized and understood. Dr. Haskins's penetrating analysis is particularly valuable here, and he does not suggest that it will be easy to heal the rifts that have grown between the kingdoms of the humanities and the natural sciences, between the bodies of thought that they comprehend and the men who are their practitioners. That the fundamental similarities, the bonds of common purpose and of common tasks unite them more strongly and more deeply than these differences are plain to see; and they offer foundations on which to build, to bridge the gaps of understanding and of sympathy, to undo the damage and eliminate potential dangers that arise. But this will not be the easier because the rifts should not exist.

First, Dr. Haskins suggests, in art coherence is primarily internal, uniting the parts of one creation. In the natural sciences, however, it is in a sense external, at its best uniting in one theoretically coherent frame the fruits of many thinkers, whose work is often extremely disparate in character. Again, in art communication and communicability extend primarily between the creator of the work and his audience, at once his witness and his critic, and such communication comes primarily after the work itself is finished. In science, on the other hand, communication is at least as active during the progress of the work as after its completion, and in a very deep sense science is fundamentally a communal activity. Though the steps of innovation lie most often with the individual, truth for science more than ever before inheres in a composite fabric of evidence and interpretation, derived from a great body of communicating investigators examining Nature from a multitude of points of view.

Truth in our day, Dr. Haskins maintains, is a property of this common fabric of accepted knowledge shared among all the working scientists in a given field, and it is for this reason that the scientific community is in essence a world community, transcending the boundaries of nations. There is probably no close counterpart to this aspect of the scientific effort within the arts; but on the other hand, while in the arts imagination alone may fix the final limits of shared experience, in science, despite the indispensable assistance given by imagination in discerning those limits and in reaching the goals they define, the limits themselves are determined by the concrete evidences of verification and therefore, ultimately, of the human senses. Nevertheless, Dr. Haskins does not omit to point out that this ultimate criterion is itself relative, joining the observer and his environment in a system from which he can never be isolated.

From this communal characteristic of science many striking contrasts between its operating modes and those of the arts are derived. One is the strong tendency to specialize, which is so characteristic of the natural sciences to-day. Dr. Haskins recognizes that specialization is essential to scientific progress; but he has wise words about the dangers which attend the consequent fragmentation of science and the need to deal adequately with the problem of communication in order that the rich opportunities which are offered by the borderlands of two or more disciplines may be explored and seized.

In this presidential report, Dr. Haskins thus ranges imaginatively and suggestively over a wide field, recognizing alike the intellectual and spiritual value of science for its own sake, its suggestive affinities with and differences from the arts and humanities, and its pragmatic importance as the architect of great material power. His review is notable for the dignity as well as the range of its thought. Dr. Haskins recognizes that it is imperative that we understand the nature and the needs of the scientific way, and comprehend the dangers that may menace it; and his review is commended to the attention of all concerned with the organization of Britain's scientific effort or the shaping of her educational institutions to meet the needs of to-day and to-morrow. They will indeed find here no blue-prints or prescriptions for policy, but they may gather some penetrating insights to the nature and needs of our larger society, some vision to inspire the leadership needed in a critical period of history the challenge of which for the future may well be, as Dr. Haskins suggests, without parallel in human experience.

PLANT MORPHOGENESIS

Plant Morphogenesis

By Prof. Edmund W. Sinnott. (McGraw-Hill Publications in the Botanical Sciences.) Pp. x+550. (New York: McGraw-Hill Book Company, Inc.; London: McGraw-Hill Publishing Company, Ltd., 1960.) 97*s*.

DURING the past twenty years, a not inconsiderable number of books and reports of symposia have been concerned with morphogenesis in plants, either from some particular point of view or in its more general aspects. The subject is in no sense new, though at different times it has been described by different names. During the nineteenth century, Naegeli, Hofmeister, Sachs, Vöchting and many others were deeply involved in the problems of the inception and development of form and structure in plants; and the present century has had some notable exponents of the subject, which now has many adherents. Yet there is justice in Prof. Sinnott's statement that few, if any, attempts have been made to treat the subject as a whole and that no extensive synthesis of its content has been undertaken. Now, after forty years as a research worker in this field, he has written the book under review—assuredly a gigantic and formidable undertaking. From the outset it is evident that he is fully aware that, in attempting a comprehensive treatment of this large subject, he is unlikely to please all his readers and that, at every point, he lays himself open to the criticisms of those with special knowledge of some particular aspect. Nevertheless, works of synthesis must be attempted by those who possess the necessary outlook, knowledge and courage, if botanical science is to retain some semblance of coherence. For this reason alone, Prof. Sinnott's book seems likely to have an important impact on botanical science.

The author has set about his task by introducing some idea of what is involved in morphogenesis, rightly emphasizing the importance of the central, if enigmatic, concept of biological organization; for at every developmental level at which there is visible expression of organic form there is evidence of unity and harmony of development, indicating the existence of co-ordinations and other inner relationships in which both genetical and extrinsic factors are involved. Part 1 of the book then begins with a consideration of growth in general, its cellular basis, and the histological constitution of apical and other meristems. In this section, also, the author has dealt, adequately and lucidly, though rather briefly, with experimental studies which have contributed to our understanding of the constitution and morphogenetic function of apical meristems. In view of the wealth of interesting illustration of apical meristems, contributed by investigators all over the world, it seems a pity, especially for student readers, that this section is so scantily illustrated.

In Part 2, the author has considered the phenomena of morphogenesis, dealing with such topics as correlation, polarity, symmetry (including the phenomena of phyllotaxis), differentiation, regeneration, chimæras and abnormal growth. This section, in short, relates to much that has been of acute interest to physiologists and others over many decades, but particularly during the more recent period. For the general botanist, and for the student of morphogenesis in particular, this section contains much fascinating and well-documented information, set forth in a simple and readable fashion.

In Part 3, the numerous and diverse factors in morphogenesis are considered, including light, water relationships, temperature, other physical factors, chemical factors in general and growth substances in particular, and of course, genetical factors. It is in this section, where a vast amount of specialized information, of very different kinds, has had to be considered and compressed into a relatively small number of pages, that the author is probably most vulnerable. He has, indeed, undertaken a task which, in view of the contemporary output of botanical research, is more appropriate to a team than to an individual. Nevertheless, he has made available to the reader a general survey of many of the factors that are involved in morphogenetic processes, and he has constantly reminded him that these factors never work in isolation: on the contrary, it is to their interaction that many of the distinctive features of living entities must be attributed.

The final chapter of the book deals with organization, obviously a topic of paramount importance in biology, but one on which few botanists have attempted to put pen to paper. However elusive and enigmatic this concept may be, its importance cannot be over-estimated. After all, some attempt to understand what constitutes organization is implicit in the aims and outlook of all those who undertake serious investigations of morphogenesis. In this chapter, which is the essence and summation of his book, Prof. Sinnott recognizes that several morphogenetic 'components' are of very wide occurrence in the plant kingdom and contribute fundamentally to the general phenomenon of organization. These components—polarity, differential gradients, symmetry and spirality—are also described as "inherent or basic tendencies". They "seem to be distinct characteristics and may have different bases in protoplasm. . . . All of them, or their rudiments, seem to be present in all plants. They provide the basic ingredients, so to speak, out of which the developmental norm is produced. Just what a specific norm will be depends on the interaction between these inherent protoplasmic traits and two other factors: the genetic constitution of the individual and the environment in which it develops". The genes, in a particular environment, act on these protoplasmic traits to produce the specific form of the organism. But, as the author points out, how genes control developmental relationships, and thus the production of organic form, is unexplored.

The author also discusses what gives unity and self-regulatory character to an organism, and concludes that ontogenetic unity, and also regenerative developments, are not so much due to the unchanging genetic constitution but to the "developmental norm, immanent in the organism from the first and often reached over different routes". In brief, the elucidation of self-regulatory, 'normative development' is regarded as the central problem in the phenomenon of organization. The difficulty of the task is indicated by the numerous workers, cited by the author, who have ventured into this field of inquiry. A synthesis of evidence from many different sources is, of course, essential, but what will come out of the attempted synthesis will, in the author's view, largely depend on the attitude and philosophic outlook of the investigator. After discussing some of the more interesting and relevant ideas that have been proposed to account for biological organization, Prof. Sinnott concludes quite simply by saying that, after all, we still have no idea of what the underlying physical basis really is. But, as is proper to one who has undertaken such an onerous task as the present book, he is not unhopeful, though he recognizes that the phenomenon of self-regulatory organization may well involve principles as yet undiscovered. These may prove to be distinctively biological and different from those at present available from the physical sciences—a conclusion which is not unlike that of some other contemporary exponents. This is a stimulating chapter, and although much of the ground that it covers is not new, it is, nevertheless, individualistic and provocative and is likely to create much new interest and discussion.

In an extensive work such as this it would be surprising if there were not grounds for criticism, of factual detail, emphasis, treatment, and so on. Some readers will no doubt find the text somewhat thin in places, for example, the treatment of embryogenesis. In the present review it is not necessary to enter into a discussion of such details. But there can be no doubt that Prof. Sinnott has produced an important and impressive book, one which seems likely to stimulate and hold its place in university teaching and research for many years to come.

C. W. WARDLAW

MODERN ARMAMENTARIUM OF THERAPEUTICS

Medicinal Chemistry

Edited by Prof. Alfred Burger. Second edition. Pp. xiii+1243. (New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1960.) 280*s*.

IT is the usual procedure that second editions of successful books are corrected versions of the original opus, with certain parts brought up to date. Prof. Alfred Burger must have faced a difficult decision when changing his role from that of sole author of the first edition to that of editor-in-chief and part-author of the new volume, at the same time re-organizing its contents. With due regard to his achievement as represented by the older version, it was a wise decision—because he has assembled around him an impressive list of contributors, holding industrial as well as academic or Government positions, but still reserving for himself the lion's share. The choice of one instead of two volumes is perhaps less fortunate as the weight of the present tome prevents one from reading certain chapters with leisure after working hours. It is also a matter for slight criticism that he selected an all-American team, which, in spite of the high standing of the individual scientists, diminishes somewhat the threads of international connexions so closely interwoven with successful research.

That does not mean that the writers on various aspects of medicinal chemistry (which, as Burger points out, can be equated with pharmaceutical chemistry and represents one very important chapter of applied organic chemistry plus pharmacology, experimental pathology and microbiology, etc.) have neglected results not originating with efforts in the United States. But with the best of intentions for fair play, a certain 'parochial' note creeps in sometimes, the reference to proprietary names, to major illnesses and pharmaceutical sales, to cholinergic instead of parasympathomimetics and to adrenergic instead of sympathomimetic drugs (Dale clearly reserved the expressions 'cholinergic' and 'adrenergic' for structures and nerves of the autonomic system). It should be said that only a very close study of the book discloses these relatively unimportant shortcomings; the overall impression is favourable, with the consequence that this volume will acquire many friends in many countries.

If a few suggestions for the third edition may be added, these are examples: an overall division into symptomatic, etiotropic (chemotherapeutic) remedies and drugs for restitutional therapy could have been given as a table and used as chapter-grouping; the historical table (2-11) has a few omissions, for example, Kennaway's name against experimental carcinogenesis and Todd's name against thiamine synthesis; action mechanism of central nervous system depressors ignores McIlwain's most vital contributions, and the difference between curare and curareform drugs and the methonium compounds is not sufficiently brought out; it is also a pity that little is said about the cannabinols mentioned only under "Analeptics", and very briefly under "Drugs for Hyperkinetic Disorders", although the synthetic derivatives represent an interesting and early group of psychopharmacological agents (formula 23-X and the name 'cannabadiol' are incorrect). Finally, an alphabetical list of authors after the subject index would have helped enormously if looking for specific references.

As said in my review of the first edition (*Research*, 4, 438; 1951) it is easy to criticize, but a very difficult task to write, such a book, and I should add even more so to edit the new version with its many improvements and attractive features.

F. BERGEL

BIRDS AND MEN IN BORNEO

The Birds of Borneo

By Bertram E. Smythies. With special chapters by Tom Harrisson, Lord Medway, and J. D. Freeman. Pp. xvi+562+99 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1960.) 84*s*. net.

THIS is a very unusual bird book. The main body of the work (about 460 pages) consists of a detailed systematic account of all the 549 species of birds that have been found in Borneo. It is written with the competence and thoroughness that distinguishes the author's previous volume on the birds of Burma. The articles dealing with the regular members of the fauna give the world distribution and Bornean status of each species, an adequate description, and discuss the habits, voice, food, and nest and eggs; the irregular visitors and casuals are treated more briefly. Appendixes contain the history of Bornean ornithology, an exhaustive bibliography, indexes of place-names, and of Malay and Iban names; and there is a large folding topographical sketch-map of Borneo. The illustrations, which are of very high quality, consist of 29 photographic plates and 50 plates in colour depicting 434 species from drawings by Commander A. M. Hughes.

But it is the hundred pages that precede this excellent treatise that put this book in a class apart. The first three chapters comprise an introduction, a topographical description of Borneo, with a discussion of the varied habitats that the island provides, and an account of bird migration in Borneo. These are followed by four chapters of ornitho-anthropology by Mr. Tom Harrisson, Lord Medway, and Dr. J. D. Freeman which are of the greatest interest. Harrisson says: "There is probably no other part of the world where birds and men are more intimately mixed than in Borneo", and goes on to discuss "Man v. Bird v. Man", "Man-Bird double-thinking" and many other matters, from Borneo's wild men and their bird values to hornbill ivory (more precious than jade), feather jewellery, and birds and the human stomach. This chapter overflows with information and ideas, and though it would gain in clarity with fewer parentheses it would lose much of its characteristic style. Lord Medway's chapter gives a fascinating account of the cave swiftlets, the saliva-built nests of which are the edible birds' nests of commerce, and which echo-navigate in the darkness of the caves where millions congregate to breed. Harrisson remarks that Governments "by some complicated zoo-geology, claim the guano as a mineral and allow extraction (for fertilizer) under licence. Thus what comes out of the swiftlets' mouth as spit is succinctly dissociated from what comes out of the other end". The chapters by Dr. Freeman cover Iban augury, "the interpretation of divine revelation as it is believed to be manifested in the behaviour of certain sacred birds", and the hornbill ritual of the Iban of Sarawak. The first part of the book is illustrated with 19 plates, two of them in colour.

The publishers are to be congratulated on the excellent standard of production, worthy of this valuable addition to ornithological literature.

L. Harrison Matthews

VEGETATION AND FOREST TREES OF GHANA

Synecology and Silviculture in Ghana

By Dr. Charles J. Taylor. Pp. xi+418+120 plates. (Edinburgh and London: Thomas Nelson and Sons, Ltd., 1960. Published on behalf of The University College of Ghana.) 100*s*. net.

THE title of this book is unfortunate, despite its euphony, because to many potential readers it will suggest something penetrating far deeper into ecological phenomena and sylvicultural practice than was ever intended, and so leave them unjustifiably disappointed. The book consists chiefly of descriptions and notes on about 160 economically or ecologically important tree-species of the Ghana rain forest. About half the letterpress devoted to each species describes its visible features; the remainder, its habitat, distribution, phenology, and, where appropriate, its sylvicultural behaviour or treatment, and its seed and seedling. The descriptions are aided by numerous photographs and drawings which doubtless account for the high cost of the book. The line drawings are less finished than many which have been published, but most of them are adequate. It is a pity that some of the drawings of seedlings are the least satisfactory (for example, *Guarea cedrata, Pterygota macrocarpa*), for it is sometimes impossible to tell from them what shape the cotyledons are, and in particular whether they are thin or fleshy. The photographs form a very useful collection, and represent an amount of work which only those with experience of photography in rain forest will appreciate.

This systematic part, which forms about three-quarters of the whole, is prefaced by a brief outline of the history, topography, soils, climate and vegetation of Ghana, excellent in its way, but somewhat disjointed and with little relevance to what follows. This general matter will be useful—but doubtfully worth £5—to students of geography or botany. Dr. Taylor writes with generally commendable simplicity, though occasionally his language becomes a little too simple for accuracy, and some ecological terms (for example, plagiosere), are used incorrectly.

The style and content of the book show that it is intended for use chiefly in Ghana. Here the book will undoubtedly be an invaluable companion to ecologists and foresters; elsewhere in the West African rain forest it will be useful chiefly for its descriptions of 'slash' and other field characters, and of seedlings. Although it is not a flora, one of its principal uses will undoubtedly be for naming trees, for which it could be greatly improved by (*a*) referring in the text to the plates, (*b*) by stressing (for example, by italics) the most useful diagnostic characters, and (*c*) by adding some kind of key or summary-table which would help the beginner in knowing which family to consult.

The book is well produced and contains very few misprints.

E. W. Jones

THE CONVOLUTION TRANSFORM

Operational Calculus

By Prof. Jan Mikusiński. (International Series of Monographs on Pure and Applied Mathematics, Vol. 8.) Pp. 495. (London and New York: Pergamon Press; Warszawa: Panstwowe Wydawnictwo Naukowe, 1959.) 100*s*. net.

THERE are many ways of developing the transform calculus, all more or less formally equivalent. Mikusiński's starting point is somewhat uncommon: the convolution of two functions $a(t), b(t)$ is

$$\int_0^t a(t-u)\, b(u)\, du$$

which he writes as ab. Since

$$ab + ac = a(b+c),\quad ab = ba,\quad (ab)c = a(bc)$$

while ab is not identically zero unless $a(t)$ or $b(t)$ is identically zero (Titchmarsh's theorem), we have an algebraic structure which is similar to Volterra's algebra of composition, and a formulation which is equivalent to Heaviside's 'algebrization' of functional operations. There are some differences, for convolutions exist over the class of continuous functions, whereas the existence of the Laplace transform requires an order condition. There is also an early difficulty, for if a and b are constants, does ab mean their arithmetic product or their convolution abt? Volterra used a special notation a^*b^*, while Mikusiński gets over the ambiguity by distinguishing the functional form $\{f(t)\}$ from the functional value $f(t)$; once one gets used to this, the calculus is simple and effective, though the reader will find it necessary at a later page to pay special attention to the introduction of operational functions.

There are plenty of applications, to electric circuits, statics of beams, vibrations, the heat and cable equations, and a good supply of exercises. Those who know the earlier Polish, Russian or German versions will appreciate the appendix of a hundred pages in the English edition, for this not only carries certain applications further, and provides on the theoretical side a link with Banach spaces, but, even more usefully, adds details to complete certain proofs which are only sketched in the main text. While perhaps the young student should stick to one line of approach to the operational calculus, his teacher should select such a line for him from a knowledge of alternatives; Mikusiński's book provides a very pleasant account, worth studying for its own sake and for comparison with more familiar formulations of this calculus.

T. A. A. Broadbent

BETTER OPPORTUNITIES IN TECHNICAL EDUCATION

By Dr. P. F. R. VENABLES

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THE second White Paper, with this encouraging title, was published in January*, nearly five years after the first White Paper on Technical Education. Much has happened in this intervening period, both in implementing the first and in determining the character of the second White Paper, and they must perforce be considered together.

The policy of the first White Paper was to establish a new four-tier structure in technical education, of local and area colleges, then regional colleges and, at the apex, the colleges of advanced technology, which were to be the main means of producing the much-needed technologists. The second White Paper directly affects the work and development of the first three levels of colleges because it contains proposals for a major reconstruction of the system of courses for technicians, craftsmen and operatives provided by them. Of the factors which have prompted these proposals, three have been dominant: The first is the need to supply more technical education for the greatly increased number of young people soon coming into employment; secondly, the need to smooth the transition from school to college; and thirdly, the need to reduce greatly the wastage in part-time courses made evident by the Crowther Report. The question is whether the proposals, and the means to implement them, are commensurate with the needs of the times.

The main proposals are summarized as follows:

(1) Students should start at a technical college immediately after leaving school. Preliminary courses in evening institutes should be discontinued.

(2) More care should be taken in the selection of students for courses. Colleges should experiment with full-time induction courses and with tutorials.

(3) The courses will include: (*a*) National Certificate and Diploma courses for students aiming to become at least high-grade technicians; (*b*) Technician courses devised specifically for particular industries; (*c*) Craft courses; (*d*) Courses for operatives.

(4) Ordinary National Certificate courses will last two, instead of three, years. The standards of entry will be raised either by insisting on four General Certificate of Education (Ordinary Level) subjects, or by passing the sessional examinations of the new general courses which will be introduced. These general courses may lead also to technician courses.

(5) Craft courses will be modified in various ways, for example, by broadening their content, and in replacing City and Guilds Intermediate by Craft Certificates; and courses for operatives should be vigorously developed.

(6) More time should be provided under day-release schemes by extending the session to the county college year of 330 hr. per annum, as specified in the 1944 Education Act, and the gain in time should be spent on general education. No student should have to rely wholly on evening study.

(7) Sandwich courses (especially for technicians) and block-release courses should be increasingly developed.

The proposals include no novelties—day-release courses are at least forty years old, sandwich courses nearly sixty years old, the county college idea more than forty years old, though the much fewer courses for operatives and block-release courses are more recent in origin. The White Paper, therefore, is to be regarded as extending the application of well-established ideas and increasing the support given to them, especially from industry. In its summary, the White Paper gives too optimistic an impression in stating that "About half a million students are attending the courses at present, and the Government look forward to a big increase in their number". A realistic basis for judging the present position and future tasks is to compare two facts: while the numbers in part-time day-release courses increased tenfold from 1938–39 to 1958–59, the numbers of students of 'County College age' still only reach about 17 per cent of the total age group (15, 16, 17 years) in employment. In short, after a ten-fold expansion over so long a time, we still have about six times as far to go 'on a voluntary basis' to include all who should be in county colleges: and by no means all the 17 per cent will be affected by this White Paper.

Significant though they are in mitigating the rigours of the part-time route, the proposals should, therefore, not be allowed to obscure the far greater advantages that would accrue to the whole age group were the recommendations of the Crowther Report implemented of both raising the school-leaving age and establishing county colleges. The White Paper admits that: "In a system of voluntary part-time courses there are limits to what can be done". With more candour, it would have inserted the word 'severe' before 'limits', for the awkward unpalatable problems remain, namely, those described by the Crowther Report as "The Need for Time" and 'The Need for Breadth". The severe limits apply both to the content of the courses and to the support they are likely to attract from industry, especially among the 73·5 per cent of 56,000 manufacturing firms each of which has less than a hundred employees.

The White Paper can, of course, only suggest a general pattern within which the many bodies responsible for the actual planning and provision of courses must do their work. Many hundreds of existing courses will need to be modified, and many new ones developed, especially for technicians and operatives. This essential task is being tackled with a commendable sense of urgency by the City and

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* Better Opportunities in Technical Education. Pp. 24. (Cmnd. 1254.) (London: H.M.S.O, 1961.) 1*s*. 3*d*. net.

Guilds of London Institute, the Regional Examining Unions, the Joint Committees and so on, and there is little doubt that these long-standing partnerships in technical education will achieve what is possible within the limited time-tables set by the proposals.

It is difficult to be sanguine as to whether the other means required are commensurate with the needs of the next decade. The first White Paper gladdened the hearts of those engaged in technical education by definitely allocating £97 million for the expansion it envisaged, and another £45 million were added in 1959, whereas no estimate of additional cost is given in the second White Paper. It might well be argued that the original target of doubling the then day-release figure of 330,000 by the mid 1960's is allowed for, or nearly so, in the building programme covered by the foregoing sums. Perhaps it is for this reason that the White Paper expresses the opinion on the cost that "relatively to the total expenditure on technical education this should be small"; and this despite the fact that the number of teachers engaged in technical education is likely at least to double in the next ten years without any effects of this White Paper being taken into account. Furthermore, the White Paper envisages a big increase in half a million students, but should this merely be up to the original 660,000, especially when attendance at evening institute courses is to be discontinued and block-release and sandwich courses are to be increased. In the latter types of courses, two students will use the space (and teaching strength) of five part-time day students with consequent increase in expenditure. The effect will be even greater in providing workshops in block-release and sandwich courses with a high proportion of practical work. In view of the sentence about expenditure quoted above, a sceptic, knowing the rearguard actions fought in the history of technical education, might justifiably conclude that the effect of the White Paper will prove to be no more than a desirable reduction of the rigours of an outmoded part-time route, supplemented by some sandwich courses, rather than the rapid replacement of it up to 18 years of age by technical education in full-time and sandwich courses. If the latter were strongly pressed forward with really adequate means instead of just bringing the past up to date, then a notable advance to meet the rising demands of the next decade would be achieved.

GOLDEN JUBILEE OF THE BIOCHEMICAL SOCIETY

By Dr. P. N. CAMPBELL and Dr. W. J. WHELAN

Honorary Secretaries

THE first meeting of the Biochemical Club was held at University College, London, on March 4, 1911. After the Club had acquired *The Biochemical Journal* in 1913 it changed its name to the Biochemical Society. This week, therefore, sees the fiftieth anniversary of the foundation of a Society instituted solely for the advancement of the science of biochemistry. We are glad to have the opportunity to survey, briefly, the past history of the Society and to look to the future.

Early Days

On reading the *History* of the Society, prepared by R. H. A. Plimmer in 1949, one is conscious of the fact that, although the Society has grown enormously, its character has altered very little. It was from the start decided that the practice of the Physiological Society should be adopted and that the Society should meet at various laboratories. During the first year, eight meetings were held, and by the time of the first annual general meeting there were 132 members. The next year was taken up with the negotiations for the purchase of the *Journal* from Benjamin Moore, the first professor of biochemistry in Great Britain, who had founded it in 1906. A poll of the members in 1912 resulted in the decision to make membership involve compulsory subscription to the *Journal*, and so the subscription was raised from 10*s*. 6*d*. to 25*s*. per annum. The Cambridge University Press has been responsible for printing and publishing the *Journal* since 1913.

In the first few years the Society made a small profit, but after the First World War the cost of publication rose and grants were received from the Royal Society and other sources. At this stage the subscription was raised, first to 35*s*. and then to 42*s*. It was many years later, in 1953, that the last change was made to 70*s*. The present sound financial state of the Society, which does not depend on any form of subsidy, is due in the first place to J. A. Gardner, who served as honorary treasurer during 1913–43.

Personalities within the Society

The early years of the Society were marked by long periods of devoted service on the part of its officers. Such extended service would not be possible to-day because of the size of the Society, which now finds it necessary to rent offices and to employ a professional staff to organize its affairs. These are only very recent developments, and for the first forty years of its existence the only premises the Society could be said to have were the offices and laboratories of its honorary officers. J. A. Gardner's long service as treasurer was matched by A. Harden's editorship of the Society's *Journal* during 1913–37.

The first chairman of the Society's committee was F. Gowland Hopkins, and his services to biochemistry were commemorated by the Society in 1958 by the endowment of the Sir Frederick Gowland Hopkins Memorial Lecture. The third of these lectures will be given by Sir Hans Krebs at the Royal Institution on March 28. The Society also shows its gratitude for outstanding service by the election of honorary members, and those so elected have been W. D. Halliburton (1923), A. Harden (1938), Sir Frederick Gowland Hopkins and R. H. A. Plimmer (1943), Sir Charles Martin (1951), Sir Henry Dale (1958) and Sir Rudolph Peters (1959).

Membership

Membership of the Society is open to all those who are interested in biochemistry, and is not confined to those who reside in the United Kingdom. In fact, more than 30 per cent of the present members live overseas. The total membership passed the 1,000 mark in 1944, and at the present figure of 3,000 has doubled in the past ten years. This means that the Society is the largest of its kind in the world.

Role in International Biochemistry

Since the Society's membership is world-wide, it is natural that it should be interested in international relations among biochemists. In 1948, the Society sent out an inquiry concerning the desirability of holding an international congress of biochemistry. The response was so favourable that the Society took the initiative and organized the first International Congress of Biochemistry, held in Cambridge in August 1949. At this time there was no international union of biochemistry, and the first Congress had the backing of the International Union of Chemistry. In this year (1961), when we look forward to attending the fifth of these congresses, to be held in Moscow in August, it is with pleasure that we recall the initiative of those who worked hard for the first congress.

After the success of the first congress, strenuous efforts were made to found an International Union of Biochemistry recognized by the International Council of Scientific Unions. These efforts were eventually successful in August 1955.

Although these large international congresses have much merit, there is also a case for holding smaller meetings on a more limited scale. The joint meetings which the Society holds with its sister societies in Europe come into this category. It has now become the custom to hold one of these meetings during the years when there is no international congress. Through these meetings, biochemists in Great Britain are able to make personal contacts with those in Europe. For our part, we offer a special invitation to European biochemists to attend the summer meeting of the Society which is held each year, either in Oxford or in Cambridge.

Meetings

The policy of holding meetings for the presentation of original contributions has changed very little since the early days of the Society. A member has the right, and is indeed encouraged, to present the results of his work before a meeting of other members of the Society. This is an important aspect of the work of the Society, and last year 260 papers were presented in this way. It is also useful, however, occasionally to discuss some particular aspect of the subject at greater length, and it was with this in mind that a series of symposia were initiated in 1947, the proceedings of which are published. So far twenty of these symposia have been held.

In addition to the two types of meeting already described, colloquia are held at which work on a particular research project is discussed. This type of meeting is normally held in conjunction with an ordinary meeting of the Society, and serves to direct attention to the work of the department being visited. The number of scientific meetings of all types reaches 404 at the fiftieth anniversary meeting.

Publications

One of the most important activities of the Society is the publication of *The Biochemical Journal*, in which are to be found many papers now regarded as classical landmarks in the development of the subject. Prof. Moore founded his *Journal* in 1906, because of his desire that contributions should be published as submitted without criticism or editorial suggestions. Much of his reluctance in parting with the *Journal* to the Society was due to his fear that this policy would be changed by the Society, and it cannot be denied that his fears were well founded. In fact, ever since the acquisition of the *Journal* in 1913 the editorial policy has been strict. This policy has helped to establish a tradition of care and proficiency in biochemical laboratories.

Within the high standard set for the acceptance of papers for the *Journal*, the policy of the Society is for the *Journal* to publish all papers submitted for publication which come within the subject of biochemistry. Authors do not have to be members of the Society. With the expansion of the subject this policy has led to an enlargement of the *Journal*, so that while in 1950 1,279 pages were published, the corresponding figures for 1955 and 1960 were 2,112 and 2,510 pages respectively. The circulation has also expanded, and there are to-day more than three thousand subscribers who are not members. This means that the *Journal* probably has a larger total circulation than any other journal devoted entirely to biochemistry.

During 1957 the committee of the Society carefully considered whether the Society should expand its publishing activities. As a result of these discussions, it was decided that clinical biochemistry was not adequately covered, and the Society eventually became the joint owners with the Medical Research Society of *Clinical Science*. This journal is now published by the Cambridge University Press, and we look to its playing an increasing part in the work of clinical biochemists in Great Britain and overseas.

The Future

In considering the future it seems likely that the activities of the Society will expand most in directions which are concerned with the status of the subject in the universities. If biochemical research is to flourish in Britain, not only shall we need to attract the best students to the departments which now have a first-degree course in biochemistry, but also more departments must be created, both for teaching and research. Last year a booklet was published on *Careers in Biochemistry*, its aim being to provide full information on training, opportunities and prospects, for the benefit of potential students and their advisers. A series of discussions is being held on various aspects of the subject. For example, at a joint meeting with the British Association, in

September 1960, a symposium was held on "The Organization and Financing of Research in Biochemistry and Allied Sciences in Great Britain". The proceedings of this discussion have now been published by the Society. Later this year, at Oxford, it is planned to discuss the problems of teaching biochemistry to undergraduates.

Mention has already been made of the recent changes in the administration of the Society's affairs, largely a consequence of the rapid growth of its membership and of the *Journal* in the past ten years. The Society's healthy financial state has enabled these changes to take place smoothly, even to the renting of premises at the very high charges prevailing in central London. Not all the necessary changes can, however, be made so easily. The size of the audiences at the Society's symposia, held in London, makes it impossible any longer to hold these in many institutions which for long have acted as hosts. It is obvious that biochemistry is in a period of rapid expansion the limit of which is not yet set, and problems of accommodation will become increasingly acute. This is part of a wider problem, and the need to take action was realized after the War, with a proposal by the Government to establish a Science Centre from which learned societies could organize their affairs, centralize information, and conduct meetings. Unhappily the proposal never bore fruit; but its fulfilment would be one of the boldest moves in advancing science in Great Britain.

The main hope for the future must be that, in spite of the expanding activities of the Society, it will be able to remain reasonably informal so that there can be a ready exchange of ideas, both scientific and administrative. By this means it is hoped that the Biochemical Society will continue to play its part in the development of a still comparatively new and exciting science.

GENE EVOLUTION AND THE HÆMOGLOBINS

By PROF. VERNON M. INGRAM

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THE four types of polypeptide chain which go to make up the molecules of the three normal human hæmoglobins are believed to be controlled by four independent genes[1]. The following article is an attempt to discuss the chemical and genetic relationship between these chains from an evolutionary point of view; to a lesser extent, because much less is known, the hæmoglobins of other vertebrates will also be mentioned. It should be emphasized that the main purpose of this discussion, and of the evolutionary scheme to be proposed, is to provide a basis for the discussion of the evolution of genes in general and hæmoglobin genes in particular. Questions are raised which will, in part, be answered soon by the chemical work on vertebrate hæmoglobins which is now proceeding in various laboratories (see, for example, ref. 2).

The study of the chemistry of the human hæmoglobins has already provided evidence of the kind of phenotypic effects to be expected from gene mutations as, for example, in the known single amino-acid substitutions in the peptide chains of abnormal hæmoglobins[1]. It is on such changes in protein structure that the forces of natural selection are assumed to act. From the evolutionary and the practical point of view it is convenient to study a commonly occurring protein, such as hæmoglobin, which is found in all vertebrates. Hæmoglobin is not only accessible for chemical study but is also sure to have played an important part in vertebrate evolution, because of its vital physiological function as the carrier of oxygen.

Most of the discussion which follows will centre around the chemical findings in the human hæmoglobins, since they are by far the best studied of vertebrate hæmoglobins. However, the basic similarities in chemical structure between man's hæmoglobins and those of other mammals and lower vertebrates are very striking[3]. All known vertebrate hæmoglobins—except those of the lamprey and the hagfish—are built on the same molecular pattern: they consist of four polypeptide chains of roughly 17,000 molecular weight, with one hæm group each. The hæmoglobin of the lamprey is peculiar in consisting of a single polypeptide chain of molecular weight 17,000. The hagfish hæmoglobin appears to be similar, or possibly a dimer of 34,000 molecular weight[3].

The peptide chains in the other vertebrates are of two different types; for example, human adult hæmoglobin consists of two so-called α-chains and two β-chains. There is usually interaction between the four hæm groups in their reaction with molecular oxygen; in other words, a plot of the degree of oxygenation of the hæmoglobin molecules against the partial pressure of oxygen is sigmoid in shape[4]. This fact enhances enormously the physiological efficiency of the hæmoglobin molecule as our oxygen carrier[5], since it favours complete saturation with oxygen in the lungs and complete discharge in the tissues. This great similarity which characterizes most vertebrate hæmoglobins suggests that we are studying that aspect of the evolution of a particular protein molecule which is concerned with detailed development of an already well-defined molecule. It is true that the hæmoglobin quadruples its size during this evolution (if we include the lamprey hæmoglobin), but the change is due to the aggregation of four fairly similar protein sub-units (peptide chains), rather than to an actual lengthening of a molecule. In the evolutionary scheme to be discussed it is suggested that the increase in complexity, and in diversity, of the hæmoglobins is an illustration of a more general process of gene evolution which results in an increase of the number of genes.

Human Hæmoglobins

The following striking situation is found in the human hæmoglobins:

(1) The three normal human hæmoglobins all possess a common half-molecule which may be written $-\alpha_2{}^A$. This formulation indicates that this half-molecule unit is composed of two normal α-peptide chains as first described for normal adult hæmoglobin *A*. However, the other half of each of the three hæmoglobins consists of different types of peptide chain, β, γ or δ, one type for each hæmoglobin. All four chains are of roughly equal size with a chain molecular weight of about 17,000. These three hæmoglobins may be formulated as follows:

Adult = hæmoglobin A = $\alpha_2{}^A\beta_2{}^A$
Fœtal = hæmoglobin F = $\alpha_2{}^A\gamma_2{}^F$
Kunkel's minor component[6] = hæmoglobin A_2 = $\alpha_2{}^A\delta_2{}^{A_2}$

This list may have to be extended as the study of other minor hæmoglobins continues[7].

I postulate[1] that four genes are involved in the manufacture of these peptide chains, so that the *genotype* of a normal individual may be written as:

$$\alpha^A/\alpha^A,\ \beta^A/\beta^A,\ \gamma^F/\gamma^F,\ \delta^{A_2}/\delta^{A_2}.$$

In this view the products of the α-genes are common to all the human hæmoglobins.

(2) In total amino-acid composition[8] the four types of chains compare as follows:

α^A and β^A — differ in perhaps 21 out of nearly 140 amino-acids
γ^F and β^A — differ in perhaps 23 out of nearly 140 amino-acids
δ^{A_2} and β^A — differ in less than 10 amino-acids.

In addition, γ^F is the only one of these chains to contain the amino-acid *iso*leucine—four residues per chain.

(3) The α^A-chain begins with the amino-acid sequence[7] Val-Leu- . . . ; the β^A-chain and the δ^{A_2}-chain begin with Val-His-Leu . . . . The γ^F-chain begins with the sequence Gly-His-Phe-, which although different from the β-chain shows a clear affinity in the type of amino-acid with the beginning of the β^A-chain. In both cases, the first amino-acid is neutral and aliphatic, the second is the basic amino-acid histidine, and the third has a non-polar side-chain.

The peptide chain beginning with the Val-Leu-sequence (α-chain) has a counterpart[3] not only in all known mammalian hæmoglobins but also in the hæmoglobins of all the lower vertebrates so far examined, except for the hæmoglobin of the lamprey. This is not to say that the Val-Leu- chains of other animals resemble the human chain precisely, but rather that there might be a strong 'family resemblance'. It is of interest to attempt to explain the repeated occurrence and strange similarity of the Val-Leu- (the α) chain in the vertebrates.

The following postulates are used in developing a scheme for the evolution of the hæmoglobin genes.

Postulate 1. Mutations of a gene result in either single or multiple amino-acid substitutions in the peptide chain which that gene controls, or inversions of part of the amino-acid sequence, or a combination of these possibilities. The new hæmoglobin peptide chains produced by such mutations are then either favoured or discarded in the course of natural selection.

Postulate 2. At several points in the course of evolution a gene for a particular hæmoglobin peptide chain has undergone *duplication*, followed by, or simultaneous with, translocation. The two initially equivalent genes have then evolved independently, governed by the selective pressure of their environment on their protein products. Such mechanisms have been previously postulated; for example, ref. 9. The role of gene duplication[9,10] in evolution has been discussed by Stephens[11]. His conclusion was that the case for duplication as an important factor in evolution was so far neither proved nor disproved.

It is of course equally possible to postulate the occurrence of chromosome duplication, with subsequent independent evolution of the initially identical chromosomes. Such a scheme would fit into the proposed hypothesis equally well.

It seems likely that α-, β- and γ-genes are located on different chromosomes, since they segregate independently, whereas β- and δ-genes appear to be linked[12].

Postulate 3. Once the α-chain, or rather the α_2-dimer, is required to fit precisely with at least two other dimers—β_2 and γ_2—the α-chain is no longer as free to be varied by mutation. It has become 'conservative' and its rate of evolution will be less than that of the other chains, as is perhaps seen in the persistence of the Val-Leu- beginning of the α-chain of the vertebrates.

In addition, one can postulate that mutations of the α-gene produce mutant α-chains which are more severely selected against in the later stages of evolution than are mutants of other chains. This is likely to be so, because in the later stages of evolution the α-chains participate in the formation of fœtal hæmoglobin, a vulnerable point in the life-cycle of the animal. It is a fact that mutants of the human α-chain never reach a high frequency of distribution, in contrast to some β-chain mutants, although we know of as many different kinds of α-chain mutants as of β-chain mutants.

Scheme for the Evolution of the Hæmoglobin Genes

We might suppose that originally the hæmoglobin molecule was rather like the present-day myoglobin molecule, that is, that it had a single peptide chain with a single hæm group, and that therefore it could not show hæm–hæm interaction. The size of this molecule might vary, but eventually it might be stabilized at a value of around 17,000 molecular weight.

At this stage of evolution, presumably earlier than the teleost fishes, the hæm protein inside the muscle cells is assumed to be the same as that in the circulation. The muscle hæm protein became myoglobin in the course of evolution; it retained a molecular weight of 17,000 and a complexity of only one hæm group and one peptide chain per molecule. It was, of course, still subject to mutational changes, as can be seen from the fact that its present-day amino-acid composition and sequence in a given animal often differs considerably from that of any of the analogous hæmoglobin chains[13]. For example, human myoglobin contains *iso*leucine, but no cysteine, whereas the reverse is true of human adult hæmoglobin.

On the other hand, one can foresee limits to the kind of mutations which would be tolerated. The X-ray studies of Kendrew[14] and of Perutz[15] show that the three-dimensional arrangement of chains in myoglobin and the hæmoglobin sub-units (also of

17,000 molecular weight) are remarkably similar, though not identical. This statement applies also to the two kinds of sub-units found in hæmoglobin itself. Presumably, mutational alterations in the course of evolution which would drastically affect the three-dimensional structure were not tolerated. Such considerations imply that the configuration of the peptide chains in myoglobin and hæmoglobin became stabilized early in evolutionary history, at least in its most important features.

During the evolution of the primitive hæmoglobin chain—provisionally called the 'α'-chain—there occurred a gene duplication followed or accompanied by translocation. From now on the two duplicate 'α'-chain genes could evolve independently—one to become the modern myoglobin gene, the other to become the α-chain gene of present-day hæmoglobin. Eventually, according to the scheme, the α-chain gene would evolve in such a way that its product, the α-chain, had the property of dimerization in solution to form α_2 molecules. Such a property would be favoured strongly, if it entailed, in addition, the possibility of hæm–hæm interaction between the two hæm groups of the new dimer molecule and therefore the possibility of more efficient oxygenation and deoxygenation. Once produced, such a mutation is unlikely to be lost in the further evolution of hæmoglobin.

At this stage, the sequence of the 'α'-chains is still variable within the dictates of structural requirements, since there is nothing yet to put additional restrictions on it.

We might next postulate that the genes of the α_2-chains duplicated again. After this gene duplication two types of dimer—α_2 and γ_2—would evolve side by side. Sooner or later, these chains would have evolved sufficiently to be able to form tetramers with even greater selective advantage because of the increased hæm–hæm interaction likely to be found in such tetramers. The characteristics of the genes responsible for the ability of the chains to form tetramers would certainly be fixed. This stage of hæmoglobin evolution seems to have been reached already in some teleost fishes, because they already possess a four-chain hæmoglobin[3].

The third gene duplication and translocation is pictured as occurring with the γ-chain gene, giving rise to a new γ-gene destined to evolve into the β-chain gene. At this gene duplication the property of forming tetramers is already firmly established. The new gene can develop along its own line to provide a hæmoglobin tetramer—$\alpha_2\beta_2$—particularly adapted for the adult body. On the other hand, the old γ-chain continues to develop and to provide half the molecule of the fœtal hæmoglobin ($\alpha_2\gamma_2$). It is the γ-chain gene, rather than the α-chain gene, which is said to duplicate here, because the γ-chain dimers, γ_2, have already the necessary complementariness for forming tetramers with α_2. This complementariness will be automatically a property of the product of the new gene. In addition, we shall see later that β- and γ-chains are more closely related to one another than either is to the α-chain. Therefore we might consider them to have diverged at a later stage.

At this point of evolution, three independent genes—α, β, γ—are assumed to be present, each one capable of forming chains which dimerize and which aggregate to the tetramers $\alpha_2\beta_2$ or $\alpha_2\gamma_2$. Hæm–hæm interaction is strongly present in the tetramers. Such a situation has an important effect on the further evolution of the α-chain. This chain, or rather its dimer, α_2, is required to fit with two different partners, β_2 and γ_2. As a result, less variation is allowed to the α-chain; it has become 'conservative'. Perhaps such conservation is in part responsible for the apparently universal presence of α-chains beginning with Val-Leu- in the hæmoglobins of the higher vertebrates. There is an alternative explanation for the apparently greater stability of the α-genes; this gene controls also the fœtal hæmoglobin and therefore may not undergo extensive mutational alterations, since the fœtus is a much more delicate organism. The very fact that any alteration in the α-chain gene, and therefore in the α-chain, seems to affect all types of hæmoglobin may be sufficient to explain the apparent 'conservatism'.

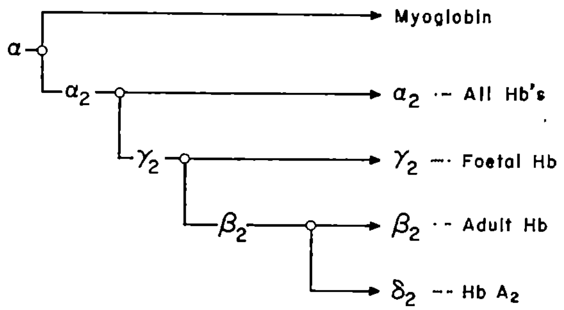

Fig. 1. Evolution of the hæmoglobin chains. The α-chain is the ancestral peptide chain. —○— indicates a point of gene duplication followed by translocation of the new gene

On the other hand, there is no *a priori* reason why different parts of a molecule as complex as a protein should develop at the same rate. The difference in apparent stability between the α-chain and the others could be no more than what would be expected as normal variation. It will be interesting to see just how similar the Val-Leu- chains of different vertebrates are.

At the fourth and last gene-duplication in the scheme we suppose that it is the β-gene which becomes duplicate, leading to the δ-chain genes controlling the δ-chains of hæmoglobin A_2. The origin of this δ-chain is placed near the end of the evolutionary scheme, because of its great chemical similarity to the β-chain[16]. Furthermore, the presence of a hæmoglobin A_2-like component seems to be confined to the higher primates[17]. In this view, hæmoglobin A_2 is a new hæmoglobin rather than an archaic one, as has often been supposed. It has been reported to have a higher affinity for oxygen[18] and perhaps it is a more efficient hæmoglobin, destined to replace eventually $\alpha_2\beta_2$ (ref. 19). Its proportion is certainly doubled in some thalassæmias[17], perhaps as a compensating mechanism. There is genetic evidence that the genes for δ- and for β-chains are linked[12], indicating perhaps that the process of translocation of the new δ-gene has not yet occurred.

Where does the lamprey fit into this scheme? One form, *Petromyzon planeri*, has two fœtal and two (different) adult hæmoglobins[20]. The molecular weight of the adult mixture is given as 17,000 (ref. 3). Perhaps these lamprey hæmoglobins are the result of an independent evolution scheme similar to the one discussed in the present article, but which has never included the mutations which led to the formation of dimers and tetramers. We might regard the lampreys as having branched off before or just after

Table 1. COMPARISON OF THE N-TERMINAL SEQUENCES OF THE α- AND β-PEPTIDE CHAINS OF HUMAN HÆMOGLOBIN A.[21]

α-chain Val-Leu-Ser-Pro-Ala-Asp-Lys-Thr-Asp-Val-Lys-Ala-Ala-Try-Gly-Lys-Val-Gly-Ala-His-Ala-Gly-Glu-Tyr-Gly-(Ala,Glu)-Ala-Leu-Glu-Arg-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Met-Phe-Leu-Ser-Phe-Thr-Pro-Thr-Lys-
32 33 34 35 36 37 38 39 40

β-chain Val-His-Leu-Thr-Pro-Glu-Glu-Lys-Ser-Ala-Val-Thr-Ala-Leu-Try-Gly-Lys-Val-Asp-Val-Asp-Glu-Val-Gly-Gly-Glu-Ala-Leu-Gly-Arg-Leu-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

the first gene duplication in the scheme of Fig. 1. Unfortunately, nothing seems to be known about the presence or absence of a separate myoglobin in the lampreys.

Discussion

The proposed scheme expresses a desire to explain the striking chemical similarity between α, β, γ and δ human hæmoglobin chains in terms of their evolution from a common precursor.

Braunitzer *et al.*[21] have published some preliminary findings of the amino-acid sequences in the first 30–40 positions of the human α- and β-chains (Table 1). Between the two sequences there is a strong 'family resemblance' of the degree expected for two gene products which have evolved independently, but from a common ancestor. Also Gratzer and Allison[8] have very recently discussed the possibility that the hæmoglobin chains might have evolved from a common ancestor.

It has been suggested to me that the four genes controlling the four chains might have evolved from four unrelated genes which originally controlled the synthesis of quite unrelated proteins; thus there would have been no increase in the total number of genes. By a process of parallel evolution—successive and independent mutations—each of these four genes eventually changed so that it made one of the hæmoglobin peptide chains. The last stages of evolution, when pairs of genes were involved in producing parts of a complex molecule, must then be called on to produce similarity in chemical structure and in configuration. It is easy to conceive of a selective mechanism which would favour changes in various members of a molecular aggregate, such that the members might fit together better. However, it is hard to think of such a mechanism also selecting for chemical and structural similarity of the monomers. In addition, the ability to dimerize would have to arise *de novo* four times, an unlikely situation.

Although we cannot at the moment completely reject the idea of parallel evolution of the four genes from unrelated genes, it is not a very palatable one. The similarity between the human α-, β-, γ- and δ-chains is so great: the C-terminus of the chains ends with the sequence -tyrosyl-histidine or -tyrosyl-arginine[16,22]; their common ability to incorporate an active hæm group; their similar folding and chain length[14,15]; absence of *iso*leucine in α, β and δ[8,16]; and so forth. Altogether, an evolution from a common ancestor is more attractive.

Chemical Evolutionary Relationship Between the Chains

How many mutational steps occurred between the original 'α'-gene and the present-day human α-gene? Or between the original 'α'-gene and the human β-gene? It is more meaningful, perhaps, to ask how many mutational events might separate the present-day human α- and β-genes. Out of 140 amino-acids, we know from the composition that approximately 20 are different. This gives a minimum of 20 mutations of the kind effecting single amino-acid substitutions.

It is believed that the evolution of the vertebrates began some 5 × 10^8 years ago. Let us assume a mean generation life of 5 years among the vertebrates, which implies some 10^8 generations for the evolution of vertebrate hæmoglobins. A generally acceptable rate[24] per generation is 10^{-5}–10^{-6}, which leads to a figure of 100–1,000 mutations in the evolutionary history of a vertebrate hæmoglobin. This figure, which is itself a minimum one, is very considerably higher than the (minimum) number of mutations which appear to distinguish α-chains from β-chains and α-chains from γ-chains.

The only conclusion which can be drawn from such a calculation is that a more than sufficient number of mutational events have passed in the history of the vertebrates to account for present-day differences between the various parts of the hæmoglobin molecule in the human species.

Comparison of the Fingerprints of the Human α-, β-, γ- and δ-Chains

Rather than deduce the chemical relationship between the chains from their amino-acid composition, it is better to compare their 'fingerprints'[24]. The two-dimensional maps, or fingerprints, of the tryptic peptides derived from each of these four peptide chains give at least a crude idea of the similarity and dissimilarity in their amino-acid sequences and hence of the closeness of the chemical relationship between them.

Human α-Chains and β- or γ-Chains

With the exception of peptide number 21 (ref. 25), all the peptides (some 14 for each chain) occupy different positions on the fingerprints[23] of α-chains compared with β- or γ-chains, or they can be distinguished by such methods as extended paper ionophoresis. They are therefore different in their amino-acid sequences. Peptide 22 is free lysine. It does not follow that the differing peptides of the α- and of the β- or γ-chains are totally altered. They could contain short amino-acid sequences in common[21]. In any event, the indications are that the amino-acid differences between the α- and the β- or γ- chains are distributed throughout their length.

The general comment might be made that there is a familial likeness between the distribution of peptides on the fingerprints of α-, β- and γ-chains. Surely this will reflect a structural relationship (Table 1). We would suppose that portions of their amino-acid sequences are common to all three, as is the tertiary structure of the two types of chain of horse hæmoglobin, which correspond to the human α- and β-chains.

Human β-Chains and γ-Chains

Apart from peptides number 21 (a pentapeptide) and 22 (free lysine), the following peptides[25] occupy very similar positions[22] and would be closely related, if not identical: peptides 12, 14, 15β, 19, 20β, 26. The number is an impressive proportion of the whole chain. If it is correct that these peptides are indeed identical in the β- and γ-chains, it would mean that perhaps 9 peptides out of some 14 are shared by the chains. Some of these peptides are quite long. Their position along the chains is not known, except that peptide 26 is the third one from the N-terminus[21]. These conclusions underline the chemical similarity between β- and γ-chains, which can also be deduced from their amino-acid compositions.

Human β-Chains and δ-Chains

The recent work of Stretton[16] has shown that the β-chains and the δ-chains are very closely similar in their fingerprints. In fact, out of a total of some 14 tryptic peptides, only 4 peptides are different; they are numbers 5, 12, 25 and 26. The similarity between other pairs of peptides from the β- and δ-chains has been ascertained much more carefully by amino-acid analysis than has so far been done with the γ-chains. Moreover, the differences between peptides 12, 25 and 26 from β- and from δ-chains have been shown to involve only 4 single amino-acid differences, for example, serine-threonine (in two places), threonine-asparagine, glutamic acid-alanine. These four single amino-acid differences, together with an unconfirmed fifth one, are so far the only ones definitely known between this pair of chains, each of which is some 140 residues long—a very close chemical similarity indeed.

Apart from the tryptic peptides of the α-, β-, γ- and δ-chains, there is in each case the trypsin resistant 'core' to be considered which amounts to rather more than a quarter of the chain. Here the differences between the chains are far from clear and cannot yet be usefully discussed, except to say that the 'cores' all appear to be similar.

Fingerprints of hæmoglobins from other mammals (ref. 2, and Muller, C. J., unpublished) are recognizable as 'hæmoglobin'. It will be interesting to compare the degree of similarity between the mammalian hæmoglobins on one hand with, say, chicken or teleost hæmoglobin.

Recently, Zuckerkandl[2] has shown that fingerprints of hæmoglobin from the gorilla and chimpanzee are indistinguishable from those of human hæmoglobin. A greater number of differing peptide spots are observed in the less closely related orang-utan and rhesus monkey. These extraordinarily interesting findings give added proof of the validity of using a chemical study of vertebrate hæmoglobins to discover evolutionary relationships.

Conclusion

It appears that a sufficient number of generations has passed since the beginning of vertebrate evolution to allow for the known or suspected number of mutations which separate the α-, β- and γ-chains of human hæmoglobin.

The similarity in the fingerprints between β- and γ-chains of human hæmoglobin supports the suggestion that these chains diverged from one another at a later stage in evolution than either from the α-chain gene.

The close similarity between β- and δ-chains gives weight to the idea that the δ-chains of hæmoglobin A_2 are a recent evolutionary development from the β-chains.

The suggestion is made that a single primitive myoglobin-like hæm protein is the evolutionary forerunner of all four types of peptide chain in the present-day human hæmoglobins, and of the corresponding peptide chains in other vertebrate hæmoglobins. Such a scheme involves an increase in the number of hæmoglobin genes from one to five by repeated gene duplication and translocations; the scheme may thus illustrate a general phenomenon in gene evolution.

I acknowledge the many stimulating discussions with Drs. C. Levinthal, S. E. Luria, C. Baglioni, A. O. W. Stretton, J. V. Neel and P. S. Gerald. I am also grateful to Drs. W. B. Gratzer and A. C. Allison for allowing me to read their review paper[3] before publication.

Two books have been particularly stimulating: J. B. S. Haldane, *The Biochemistry of Genetics*; and C. B. Anfinsen, *The Molecular Basis of Evolution*.

No serious attempt has been made to survey critically the literature on gene evolution or duplication. This is partly in order to present a clearer and more provocative hypothesis and partly because I do not feel qualified to evaluate the numerous contributions to that field. Recent experimental work on the animal hæmoglobins has been admirably summarized in the Gratzer and Allison article[3].

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Note added in proof. Further work by G. Braunitzer and his colleagues (*Z. physiol. Chem.*, in the press) shows that the similarity of the α- and β-chains continues throughout these chains.

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OBITUARY

Prof. J. T. Patterson

John Thomas Patterson, emeritus professor of zoology in the University of Texas, who died on December 4, 1960, at the age of eighty-two, will be remembered especially for his work on evolutionary genetics of *Drosophila*, published as a series of "Studies in the Genetics of Drosophila" by the University of Texas and culminating in a book, *Evolution in the Genus Drosophila* (1952), written in collaboration with Wilson S. Stone. Yet this great programme of work, involving the activities of numerous colleagues and graduate students, was initiated after he reached the age of sixty, in the evening of a long life devoted to biological teaching and research.

J. T. Patterson was born at Piqua, Ohio, on November 3, 1878. He received his bachelor of science degree from the College of Wooster in 1903 and his Ph.D. from the University of Chicago, where he was a student of C. O. Whitman, in 1908. He went to the University of Texas in Austin in 1908 as instructor in zoology, becoming adjunct professor in 1911, professor in 1913, 'distinguished professor' when this special rank was created in 1937, and emeritus professor on his retirement from teaching (but not from research) in 1955. He was one of the last of a great generation of Chicago Ph.D.'s in zoology that included such men as W. C. Allee, George Bartelmez, Carl Moore and Oscar Riddle.

When Patterson arrived at the University of Texas he found extremely primitive conditions in a frontier society. By his own efforts he built up the Department of Zoology, successively adding to the staff Carl Hartman, T. S. Painter and H. J. Muller. The bat-infested laboratory of the early years was replaced by the Biology Building in the 1920's and the magnificent Experimental Science Building in 1952. A total of thirty-one students received their M.A. degrees and twenty-nine their Ph.D.'s under Patterson's direction during the forty-eight years of his active teaching career in Texas. An extremely hard worker, he would arrive at the laboratory each morning about 6 a.m., spending the whole day in the big 'fly lab', at his microscope or talking with his students, with whom he had a very close personal relationship.

Patterson's early research was embryological. He studied the mechanism of development in the nine-banded armadillo, in which monozygotic quadruplets are invariably produced. From polyembryony in a mammal it was a natural step to studying polyembryony in the wasp *Paracopidosomopsis floridanus* Ashmead—a rolling name with which Patterson used to impress his elementary students, just as, in later years, he was able to silence an inquisitive Texas yokel by assuring him that his peculiar activities with an insect net were "collecting *Drosophila pseudoobscura* Frolova for my friend Professor Theodosius Dobzhansky".

Following on the discovery by Muller in 1927 of the mutagenic properties of X-rays, Patterson turned his attention to radiation genetics and worked on somatic mutation, mosaicism, gynandromorphs and the production of deletions and translocations by X-rays. But by the middle 1930's his active and restless mind seemed to have tired of these lines of work and for a while he devoted himself to building up a great collection of the flint artefacts of Texas and the neighbouring States, on which he published several papers. At last, in 1938, he found his greatest field of activity in the evolutionary genetics of *Drosophila*. This was a few years after Painter had shown that the salivary gland chromosomes of *Drosophila* could be used for an entirely new type of cytogenetic analysis which permitted a detailed comparison of the gene sequences of different species. The idea seems to have suddenly struck Patterson that he wanted to study, not just *Drosophila melanogaster*, or even the dozen or so other species that had been employed until then by a few geneticists, but the whole range of species belonging to this genus of flies—200, 500 or however many there might be (we now know there are at least a thousand).

Collecting expeditions were sent out to all parts of the United States and Mexico and later to Central America, Canada, the Caribbean and the Pacific area. Hundreds of species were brought back to the Texas laboratory, which became a living museum of *Drosophila* stocks which were subjected by Patterson and his students to a great variety of genetic techniques designed to elucidate their relationships. Painter's salivary gland technique was fully exploited in this work. The results were too voluminous to be published in the regular journals, so that they appeared in the now famous *University of Texas Publications*, generously distributed free to institutions and individuals throughout the world. Both taxonomy and genetics acquired new perspectives as a result of this work, for which Patterson received the Daniel Giraud Elliott Medal of the U.S. National Academy of Sciences in 1951 (he had been elected to the Academy in 1941). His studies of the *virilis* group of species will always remain classical examples of this kind of work.

Short of stature, direct and forthright in speech, Patterson impressed one with his energy, exuberance and whole-hearted dedication to his science. He never travelled outside North America and seldom left Texas after his middle years, when he still used to spend the summers with his family at Woods Hole and participate in the main stream of American biology. Thus he became something of a legendary personality even in his own lifetime to the many younger biologists of the eastern States and the west coast who had never met him. He had little use for intellectual subtleties, and his work deals almost entirely with the big problems open to a frontal attack. Although he made no single discovery comparable in importance with those of Muller and Painter, he has an assured place in the history of genetics and evolutionary biology. His influence on the development of the University where he taught and worked for more than half a century was very great.

M. J. D. White

NEWS and VIEWS

University College of North Staffordshire: Dr. H. M. Taylor, C.B.E.

DR. H. M. TAYLOR, secretary general of the Faculties in the University of Cambridge, has been appointed principal of the University College of North Staffordshire, Keele, from October 1, to succeed the late Sir George Barnes. He will bring to the service of Keele outstanding gifts of personality and a wide range of academic and administrative interests and experience. Dr. Taylor went up to Clare College, Cambridge, in 1928, after a distinguished undergraduate career at the University of Otago, New Zealand, and he continued to distinguish himself as undergraduate and research student in Cambridge. He graduated Ph.D. in 1933 and was elected Fellow of Clare in the same year. By 1939 he had made his mark in Cambridge as University lecturer in mathematics, secretary of the Faculty Board of Mathematics, director of studies in mathematics and steward of his College, and adjutant of the Officers' Training Corps. In these and many other fields he had given proof of great organizing ability, an orderly mind, industry, and a kindly and sympathetic personality.

After war service in the Army, as an instructor at the School of Artillery, Larkhill, Dr. Taylor returned to Cambridge in 1945 as University treasurer, and was made secretary general of the Faculties in 1953. He has thus had a unique experience in administering the educational and financial affairs of Cambridge, and he has made notable contributions to solving the complex problems of post-war building programmes and the academic development of the University. Dr. Taylor is in the first rank of ski-mountaineers and is also an authority on pre-Norman Conquest churches, on which he has in preparation what promises to be a definitive work. For his contributions to the study of Anglo-Saxon architecture he has recently been admitted to the fellowship of the Society of Antiquaries. To illustrate these interests he has brought a talent for photography and draughtsmanship. Despite the heavy calls of his office, he has remained readily accessible and prompt to help any member of the University staff, at whatever level, who has sought his counsel. He and Mrs. Taylor have been generous hosts, and they will leave in Cambridge many friends who will wish well to them and to Keele under Dr. Taylor's principalship.

Geography at the London School of Economics

THE constitution of the University of London is sufficiently elastic to permit the existence of many special institutions or arrangements. For the past forty years the Joint School of Geography of King's College and the London School of Economics has so flourished as to rank as the largest honours school in Britain both in numbers of staff and students. Students registered at King's College enter for degrees in the Faculties of Arts and Science, those at the London School of Economics in Arts and Economics; by tradition, though not by requirement, the professor at King's College specializes on the physical side, his colleague and joint head at the London School of Economics on the economic and human, but both are holders of University chairs of geography. As part of the post-war expansion of the social sciences, a third chair, primarily, though not entirely, a research chair, was created and occupied by Prof. L. Dudley Stamp during 1948–58, with the title of professor of social geography. Prof. Stamp was succeeded in the regular professorship and joint headship of the School by Prof. R. Ogilvie Buchanan, who was previously at University College, London (see *Nature*, 164, 475; 1949). Prof. Buchanan retires in September under the age limit after thirteen years of office, during which he has held with distinction many positions in the University—including the present chairmanship of the Academic Council.

Prof. Emrys Jones

WHEN Prof. Stamp retired through pressure of outside work in 1958, a third chair of geography was established, to which Dr. Michael J. Wise was appointed, together with a readership in social geography to which Dr. Emrys Jones was appointed. Dr. Jones now becomes the third member of the professorial triumvirate—with Prof. S. W. Wooldridge and Prof. Wise. Although the special chair or readership in social geography is now therefore in abeyance, Prof. Jones's appointment assures that the field of study, essentially the application of geographical methods of survey and analysis to present problems, will not be neglected. Trained under Prof. H. J. Fleure at Aberystwyth, he went to London after some years at the Queen's University of Belfast. His recently published book, *A Social Geography of Belfast*, is a notable contribution, breaking new ground and showing promise of new developments in the borderland between sociology and geography.

Botany at Jodhpur: Prof. C. V. Subramanian

PROF. C. V. SUBRAMANIAN has been appointed to the newly created chair in botany of the University of Rajasthan, at Jodhpur. Prior to his appointment, Prof. Subramanian had successively held the posts of lecturer (1951–53) and reader (1953–58) in botany at the University of Madras, and professor of plant pathology (1958–60) in the Indian Agricultural Research Institute, New Delhi. After graduating with a first-class honours in botany from the University of Madras in 1944, he studied soil mycology under Prof. T. S. Sadasivan in the same University, and obtained his Ph.D. in 1947 for work on the saprophytic behaviour of the *Fusarium* causing cotton wilt. In 1957 he obtained the D.Sc. degree of the same University for his work on the taxonomy of imperfect fungi. For several years past he has made an intensive study of the hyphomycetes including soil *Fusaria*, and has published many papers on the tropical forms of this group, as well as the Sphaeropsidales which had hitherto not received much attention from Indian mycologists. For some time Prof. Subramanian worked in the Botany School, Cambridge, with Dr. S. D. Garrett on soil fungi, and in the Commonwealth Mycological Institute, Kew, on hyphomycetes with Mr. E. W. Mason. Prof. Subramanian is a Fellow of the National Institute of Sciences of India and of the Indian Academy of Sciences. Although a new laboratory has yet to be constructed at Jodhpur, work has already been commenced by him on the taxonomy and biology of coprophilous and soil fungi, on the taxonomy, physiology and cytology of *Helminthosporium* species attacking cereals, and on abnormal growth in plants. B. Tiagi, appointed as reader, has a long record of active research in plant morphology and embryology. The Department will soon have a separate building together with a general herbarium of higher plants, a mycological herbarium and a collection of living cultures of fungi.

Ford Foundation Research Fellowships of the Zoological Society of London

In May 1960 the Ford Foundation made a grant of 550,000 dollars to the Zoological Society of London to endow three senior research fellowships and three junior research fellowships in animal reproduction. The Council of the Society has now appointed Dr. Peter A. Jewell, Dr. Lorna M. Kellas and Mr. D. R. S. Kirby to fellowships. Dr. Peter A. Jewell, who will hold the appointment for five years, is at present working at the Royal Veterinary College on the breeding biology of Soay sheep, which are confined to the Island of St. Kilda, and which present unique material for the investigation of a population of small ungulates. He also intends to study the reproductive performance, under laboratory conditions, of various forms of *Apodemus*. Until suitable laboratory space is available at the Society's Gardens, Regent's Park, Dr. Jewell will work in the Department of Animal Husbandry and Veterinary Hygiene, Royal Veterinary College, London. Dr. L. M. Kellas, who is appointed for three years, at present holds an appointment in the Department of Veterinary Histology, University of Glasgow Veterinary School. Dr. Kellas will make a histological and histochemical study of the intra-epithelial granular cell which occurs in the placenta of ruminants. She will also study the reproductive tracts of the giraffe, having already obtained certain material for these studies while on field expeditions to Central Africa. Mr. David R. S. Kirby has been appointed for a period of three years, as from October 1, 1960, and for the time being will continue to work in the Department of Zoology and Comparative Anatomy in the University of Oxford. He will study the effects of abnormal environments on the development of the embryo, with particular reference to the inhibitory influence of the endometrium on the spread of the cells of the trophoblast.

New Atomic Energy Authority Reactor

A new low-power reactor called *Vera* (Versatile Experimental Reactor Assembly) started operating for the first time at the Atomic Weapons Research Establishment, Aldermaston, on February 22. It is being used in reactor physics experiments to improve nuclear data and methods of calculation for fast critical assemblies. More information on these systems is required for many problems relating to the safety of storage and processing of fissile materials, and for the fast reactor development programme. The reactor includes a core region of thin-walled steel fuel tubes surrounded by a natural uranium reflector —the whole forming a cylinder 4 ft. high and 5 ft. in diameter. The fuel tubes are loaded with fissile and diluent material plates 1·7 in. square and $\frac{1}{8}$ in. thick. By appropriate loadings the composition and size of the reactor core may be varied to examine the nuclear importance of materials over a wide range of neutron energies. The reactor is designed to separate into two parts for safe loading, the two halves being brought slowly together by remote control. An accelerator associated with *Vera* enables neutron bursts to be generated for measurements of reactivity and time-dependent behaviour. The reactor is located in a large concrete cell which is sealed during operation. As the power will not exceed 100 W. special cooling is not required. This work forms part of a programme of experimental and theoretical nuclear data work at the Atomic Weapons Research Establishment and is co-ordinated with fast reactor work in progress at other establishments of the U.K. Atomic Energy Authority.

New British Base in the Antarctic

A new British Antarctic base—one of the most southerly in the chain of scientific stations maintained by the Falkland Islands Dependencies Survey—has been set up on Adelaide Island. Sir Vivian Fuchs, director of the Survey, states that the new base is on the east side of Adelaide Island, in approximately latitude 67° 40′ S. Adelaide Island is about 70 miles long and 30 miles wide with high mountains, the peaks of which reach 4,000–7,000 ft. The new base was established on February 3 and will enable the survey and geology of the Island to be completed, besides acting as a supply station from which men and stores can be flown to other bases. Two Otter aircraft have already arrived at Adelaide and are at present in the course of flying stores to Fossil Bluff, the site of a new station in lat. 71° 20′ S., long. 68° 17′ W. This will be the farthest south base in the Graham Land area, and from it exploratory journeys will be made in Alexander Island, a high mountainous region some 20,000 square miles in extent.

Afforestation in National Parks in Britain

In a written answer circulated in *Hansard* of February 14, the terms of a scheme for afforestation in the national parks in Britain is given, together with a letter sent by the National Parks Commission on January 26, 1961, to the individual park planning authorities recommending its adoption. The purpose of the scheme is to secure consultation of the park planning authorities on proposals for private afforestation of any land that has not hitherto been planted. The Timber Growers' Organization, the Country Landowners' Association, the Forestry Commission and the National Parks Commission are all anxious to secure that in the future use of land in national parks, proper regard should be given both to the needs of forestry and to the preservation and enhancement of the natural beauty of the landscape. To avoid conflict between these two interests, the parties to the agreement believe that the closest co-operation should be maintained between those concerned with afforestation and those responsible for accomplishing the purposes for which national parks have been established, and they agree accordingly that the Park Planning Authorities should be given the opportunity of commenting on all proposals for the afforestation of any land not hitherto planted. In each national park, except where special arrangements have already been made, it is proposed that the Park Planning Authority should carry out with the Forestry Commission a survey with the view of compiling maps classifying the land in the park so far as possible into: (*a*) areas where there is a strong presumption that afforestation would be acceptable; (*b*) areas where, although there is a presumption against afforestation, proposals might be acceptable; and (*c*) areas where there is a strong presumption against afforestation. These categories are devised as a general guide to policy and should be adjusted where necessary to meet particular circumstances. The surveys are intended to be discussed between the planning authorities, the National Parks Commission and the other parties to the agreement. The Forestry Commission has promised to frame its afforestation proposals in accordance with the surveys, amended as necessary after these consultations, and it is hoped that private landowners will do likewise. The arrangements will not apply outside national parks, nor to the replanting of existing woodlands. The agreement does not refer to the character or type of afforestation.

Radioactive Materials in Food and Agriculture

ALTHOUGH the references quoted in the recent report of a Food and Agriculture Organization Expert Committee, entitled *Radioactive Materials in Food and Agriculture*, and the contamination-levels available to the Committee were no more recent than 1958, and there are still wide gaps in our information, the publication represents a valuable balanced and readable general survey of the subject, and one which lays stress on basic concepts (Pp. ix + 123. Rome: Food and Agriculture Organization of the United Nations. London: H.M. Stationery Office, 1960. 7*s*. 6*d*.; 1.50 dollars). Radionuclides enter the human body, primarily via the food chain, along which a great many environmental variables can modify the contamination of the final food product. Some emphasis has been placed here on matters of soil and plant contamination, but attention is given to animal products, food processing, marine biology, human dietary aspects and overall survey and sampling problems. Although the predominant man-produced contamination of our environment with radionuclides on a world-wide scale has its origin in nuclear explosions, effluents from nuclear power-plant can be a factor of local importance. Some attention is directed to the ability of some marine forms to concentrate certain radionuclides one-hundred-fold from the sea, and to a feasible method for decontaminating milk supplies in an emergency. The Committee makes important recommendations which specify those particular fields where further research and survey work are most urgently needed. It is perhaps as well that the few errors which occur in the text so greatly alter the sense of the passage that they are easily detected; for example, on page 41, environmental carbon-14 does not amount to approximately 10–11 per cent of carbon-12.

Origin and Evolution of Coal

THE Geological Survey and Museum has published a 17-page pamphlet entitled *The Origin and Evolution of Coal* (Department of Scientific and Industrial Research. By Dr. P. J. Adams. Pp. i + 17. (London: H.M. Stationery Office, 1960.) 1*s*. 9*d*. net); this is substantially a reprint of an article that appeared in the *Fuel Economy Review* of 1955. Starting from the definition of coal as "a combustible rock which had its origin in the accumulation and partial decomposition of vegetation", it traces the history of the different vegetable ingredients that went towards the formation of coal, first through their biochemical decomposition to peat and sapropel (slimy sediment), and later through physical and chemical changes brought about by temperature and pressure during the course of the burial of peat and sapropel to greater depth. The familiar 'humic' coals, assumed to be derived from peat, together with their constituent macerals, and the sapropelic coals (cannel and boghead), are then described. Following a brief discussion of the part played in these processes by crustal movement, the lignite–anthracite series is discussed with reference to the progressive chemical changes involved. Finally, the geological record of coal formation is summarized in a useful table. Begun about 300 million years ago, this process still continues, though in small measure: for example, in the Everglades of Florida. It is thought possible that natural graphite might be derived from still earlier coals. This booklet should prove helpful to those requiring a concise picture of coal geology, for use in lecturing and teaching.

Labelling of Medicinal Preparations

A MEMORANDUM giving guidance on the procedure to be followed in devising labels and titles for compounded medicinal preparations has been issued by the Association of British Pharmaceutical Industry, the Proprietary Association of Great Britain and the Association of Public Analysts (*Labelling Procedure for Compounded Medicinal Preparations*. Pp. 6. London: Association of British Pharmaceutical Industry, 1960). Section 6 of the Food and Drugs Act, 1955, enacts certain requirements with regard to the labelling of food and drugs. When considering the provisions of the Act practitioners realize that the attitude of the public to the purchase of medicinal preparations is not necessarily identical with their approach to the purchase of food. Consequently, it may be desirable to apply to medicinal preparations labelling requirements comparable with those introduced for food by Statutory Orders and Instruments. Further, the law does not impose on medicinal preparations labelling requirements comparable with those introduced for food. The memorandum gives detailed recommendations for labelling as well as definitions of the compounds affected.

Nuclear Fusion: Journal of Plasma Physics and Thermonuclear Fusion

IT is natural, though not necessarily pleasing, that the increased interest in the physics of ionized gases should lead to the appearance of specialist journals dealing with recent developments in that subject. *Nuclear Fusion*, subtitled *Journal of Plasma Physics and Thermonuclear Fusion*, which will probably be preoccupied for some time with the former subject, is the latest addition (1, No. 1 (September 1960). Pp. 68. Published quarterly. (Vienna: International Atomic Energy Agency, 1960) Annual subscription 250 Schillings; 70*s*.; 10 dollars. Single issues 75 Schillings; 21*s*.; 3 dollars). It is a well-produced journal, with photographs and diagrams accompanying the text, although the advantage of two-column printing in a subject so mathematical as to require frequent whole-width displayed formulæ is obscure. The material in the first issue, which is of high quality, consists of the first part of a leisurely and complete survey of the oscillations of a plasma, a highly mathematical discussion of the stability of an axially symmetric plasma, a brief note on the energy distribution of thermonuclear neutrons, and two experimental papers on rapidly compressed plasmas. There is the usual impressive list of editors, and, as befits the organ of an international agency, abstracts are provided in four languages, English, French, Spanish and Russian.

Ilfracombe and District Ordnance Survey Map

THE Ordnance Survey Department has published a map at the scale of 2½ in. to the mile showing the North Devon coast from Saunton Sands to Combe Martin and extending nearly ten miles inland; Lundy Island is shown as an inset (Map of Ilfracombe and District. Sheet 856. Scale: 1 : 25,000, about 2½ inches to one mile. Chessington, Surrey: Ordnance Survey, 1961. Paper, 6*s*. 6*d*. net). Two new features have been introduced in this publication. The sheet size has been increased from the 10-km. square, at present used for Ordnance Survey maps at this scale, to 20 km. east to west by 15 km. north to south (roughly 12½ miles by 9¼ miles). In addition, to keep the price as low as possible, the cardboard cover normally used for the folded style maps has been discarded and the cover design has been printed as an integral part of the map. Covering as it does a very popular holiday and walking area, it is questionable whether the last-mentioned innovations have not, in fact, jeopardized the durability of the map, which is likely to have to stand up to some rough treatment.

Science Progress

A VALUABLE review of the giberellins by P. W. Brian, of the Akers Research Laboratories, Imperial Chemical Industries, Ltd., in the latest issue of *Science Progress* (49, No. 193; 1961) is accompanied by two other articles which should be of considerable interest to those wishing to extend their knowledge of genetics. Dr. G. H. Beale, of the Department of Animal Genetics, University of Edinburgh, describes recent work on the mechanics of cytoplasmic inheritance, while Dr. Gordon Haskell, of the Genetics Department, Scottish Agricultural Institute, reviews scientific advances in the past decade which are associated with the life-history and cyto-genetics of the genus *Rubus*. The journal also contains the usual sections describing recent developments in various branches of natural science, and many authoritative book reviews.

A Large Red-Shift

NEBULAR red-shifts up to one-fifth of the velocity of light have been reached by spectroscopic investigations. The absorption features in the spectra of even fainter and more distant galaxies are veiled by the night-sky spectrum. Emission lines remain visible for much fainter galaxies than absorption lines, and the detection of galaxies with strong emission lines would therefore permit spectroscopic determination of very large red-shifts. The investigation of radio sources offers a promising way of finding distant galaxies with emission lines; many of the strongest radio sources have been identified with such galaxies. Among the known radio sources one has now been identified with a galaxy of apparent magnitude 21, which appears to be the brightest member of a cluster of galaxies, and which has a spectrum showing a strong emission line at $\lambda 5448$ Å. The emission line is almost certainly the forbidden doublet of ionized oxygen, the laboratory wavelength of which is 3728 Å., and the large red-shift corresponds to a velocity of 0·46 times the velocity of light (R. Minkowski, *Astrophys. J.*, **132**, 909; 1960). W. A. Baum has used photo-electric photometry to determine a red-shift of 0·44 times the velocity of light for two fainter galaxies of the cluster. There is thus every reason to believe that the first galaxy is, indeed, a real member of the cluster of galaxies. These results establish the possibility of reaching red-shifts which are large enough to permit discrimination between different cosmological theories.

Scottish Field Studies Association

RECENTLY the programme for the Scottish Field Studies Association has become available and shows a welcome expansion in the scope of its courses, of which seventeen are now being offered. The majority of the botanical courses are once more centred on the Garth Field Centre. However, other courses are being organized at: the Isle of Raasay and the Isle of Great Cumbrae (marine biology); the Isle of Arran and North Ballachulish (geology); Beinn Eighe, the Isle of Arran and Strathpeffer (zoology). Two geographical field studies are being arranged from the Garth Field Centre and in the Strathpeffer region. Fees for the courses, which in each case last a week, vary from £7 to £11 3s. Further information can be obtained from the Scottish Field Studies Association, 179 West Regent Street, Glasgow, C.2.

Institute of Metals

THE spring meeting and annual general meeting of the Institute of Metals are to be held at Church House, Great Smith Street, London, S.W.1, during March 21–23. The annual general meeting will be held on the morning of March 21. On each occasion the programme is divided into two sections, *A* and *B*, which will run concurrently in the Hoare Memorial Hall and the Convocation Hall, as follows: *March* 21 (*A*) compatibility problems in gas-cooled reactors, (*B*) aluminium alloys (Prof. M. Polanyi will be giving the May Lecture, entitled "Science: Academic and Industrial", in the evening); *March* 22 (*A*) copper alloys and X-ray fluorescence analysis, (*B*) the contribution of constitutional research to general metallurgical problems; *March* 23 (*A*) extrusion, (*B*) segregation to grain boundaries. Further information can be obtained from the Secretary, Institute of Metals, 17 Belgrave Square, London, S.W.1.

International Congress on Metallic Corrosion

THE first International Congress on Metallic Corrosion is to be held at the Imperial College of Science and Technology, London, S.W.7, during April 10–15. The programme has been divided into fifteen sessions which include such topics as effects of corrosion, corrosion inhibitors, corrosion protection, high-temperature oxidation, corrosion fatigue, etc. It will also include four plenary lectures: "Chemical and Electrochemical Behaviour of Metals at Passivation Ranges", by Prof. Y. M. Kolotyrkin; "Radio-tracers as applied to the Study of Metallic Corrosion", by Prof. P. Lacombe; "The Advancing Frontiers of Corrosion Science", by Prof. H. H. Uhlig; "Factors deciding between Active Corrosion and Protective Film Formation", by Dr. U. R. Evans. Further information can be obtained from the Honorary Secretary, The First International Congress on Metallic Corrosion, 14 Belgrave Square, London, S.W.1.

Teyler Essay Competition

THE 1961 competition for the Teyler Prize, organized jointly by the Teyler Foundation and the Second Teyler Society, will take the form of an essay on modern theories of the genesis of organic matter and macromolecules originating during a phase of development of the Earth, prior to the appearance of the first living organisms. The Teyler Prize consists of a gold medal or 400 Dutch guilders. The closing date for the competition is January 1, 1963. Further information can be obtained from the directors of Teylers Stichting, Fundatiehuis, Damstraat 21, Haarlem (Netherlands).

University News:

Belfast

THE following appointments to lectureships have been announced: B. E. S. Gunning (botany); Dr. W. D. McGrath (physical chemistry); B. C. S. Wilson (archæology). Grants have been received as follows: from the Department of Scientific and Industrial Research, £695 for the purchase of an argon chromatograph, and £768 for the purchase of a gas chromatograph with flame ionization detector, both for research in the Department of Chemistry; from the Wellcome Trust, £5,500 for the purchase of an Akashi electron microscope and an ultramicrotome for use in research by the Department of Zoology.

Birmingham

AMONG the recent appointments are the following: J. W. Fortey, to be lecturer in civil engineering; D. E. N. Davies, to be Associated Television Fellow in the Department of Electrical Engineering; B. Henderson, to be William Gibbins Research Fellow in the Department of Physical Metallurgy; Dr. A. I. Rees to be Imperial Chemical Industries Fellow in Geophysics in the Department of Geology.

Keele

THE Nuffield Foundation has given £20,000 to the University College of North Staffordshire for the purpose of setting up and maintaining a special library, to be known as the Nuffield Library, appropriate for the foundation year of general studies in the College. This Library, to be housed in the new University Library building under construction, will contain multiple copies of a number of texts which are basic to the study of each of the twenty or more subjects which go to make up the foundation year course. Part of the grant is to be available to keep this Library constantly up to date.

London

DR. F. F. HEYMANN has been appointed reader in physics in the University of London, and Dr. J. Lewis reader in chemistry in the University of London, in respect of posts held by them at University College.

Sheffield

THE following appointments have recently been announced: Dr. S. C. R. Dennis, senior lecturer in applied mathematics; Dr. R. C. Hewson-Brown, lecturer in applied mathematics; Dr. D. S. Dugdale, Speedicut research fellowship in mechanical engineering; J. Beech, United Steel Companies' fellowship in metallurgy; D. H. Warrington, J. H. Andrew fellowship in metallurgy; A. Mitchell, C. H. Desch research fellowship.

Announcements

PROF. J. D. BERNAL, professor of physics in Birkbeck College, University of London, has been elected a member of the Czechoslovak Academy of Sciences, in recognition of his outstanding contribution to scientific knowledge in the field of physics.

SIR THOMAS SCRIVENOR, deputy high commissioner for Basutoland, the Bechuanaland Protectorate and Swaziland, has been appointed secretary of the Commonwealth Agricultural Bureaux.

THE third meeting of the Biological Engineering Society is to be held in Bristol on April 29. The Society will be visiting the University, in particular the Department of Anæsthetics and the Burden Neurological Institute. Further information can be obtained from Dr. A. Nightingale, Physics Laboratory, St. Thomas's Hospital, London, S.E.1.

A GENERAL meeting of the Association of Applied Biologists is to be held at the Manor House, Rothamsted Experimental Station, Harpenden, on March 10. The meeting will include a symposium on "Physiology of Field Crops". Further information can be obtained from Miss G. N. Thorne, Rothamsted Experimental Station, Harpenden, Herts.

A ONE-DAY symposium on the application of interference microscopy to problems of research is to be held by the Royal Microscopical Society on March 15 in its rooms at Tavistock House South, W.C.1. Further information can be obtained from the General Secretary, The Royal Microscopical Society, Tavistock House South, Tavistock Square, W.C.1.

THE fourth symposium in agricultural meteorology will be held in the University College, Aberystwyth, on March 8. The topic for discussion is "Soil Climate with Special Reference to the British Isles". Particulars can be obtained from Mr. James A. Taylor, Department of Geography and Anthropology, University College, Aberystwyth.

THE fourteenth Laboratory Animals Centre Congress is to be held at the University of Bristol during April 10–11. Admission to the Congress is by invitation only. Forms of application and further information can be obtained from the Director, Laboratory Animals Centre, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey.

THE Third Australian Spectroscopy Conference will be held in the Chemistry School, University of Sydney, during August 22–24, under the sponsorship of the Standing Committee on Spectroscopy of the Australian Academy of Science. The Conference will cover all aspects of pure and applied spectroscopy including the newer fields such as resonance spectroscopy. Further information can be obtained from Dr. L. E. Lyons, Department of Physical Chemistry, University of Sydney.

GEOCHEMICAL LABORATORIES, LTD., 2 Elmfield Avenue, Teddington, Middlesex, announce that these Laboratories, hitherto carried on in partnership by Mr. H. B. Milner and Mr. A. H. Clarke, will be continued by Mr. Clarke under the same name. Mr. Milner will continue to be actively concerned with the Laboratories and has agreed to act as consultant and adviser.

RECENT DEVELOPMENTS IN PLANT NEMATOLOGY

THE Association of Applied Biologists held a symposium on "Recent Developments in Plant Nematology" on January 20 at the British Museum (Natural History). The symposium was well attended and appears to have been appreciated.

Dr. H. C. Gough (National Agricultural Advisory Service, Cambridge), speaking on "The Importance of Plant Parasitic Nematodes in Britain", pointed out that the root nematodes, species of *Heterodera*, are the most serious pests. *H. rostochiensis* infests at a detectable level more than 80 per cent of potato fields in Holland, Lincolnshire, and about 40 per cent in the Isle of Ely ; about 10 per cent of the total potato acreage in eastern England is infested badly enough to cause serious crop loss. In the case of glasshouse tomatoes, the pest often has to be controlled by costly steam sterilization. *H. schachtii* infests about 2 per cent of the total beet-growing area, and a sugar beet eelworm order was introduced in 1943 to reduce the spread and build-up of the pest by control of cropping. Pea root eelworm *H. göttingiana* could well become a serious pest in the rapidly expanding vining pea industry. Stem eelworm, *Ditylenchus dipsaci*, is most serious on narcissus and tulips in the bulb-growing areas. On narcissus, it is controlled to a considerable extent by regular hot-water treatment, but on tulips no similar satisfactory treatment has yet been devised. Stem eelworm in oats is fairly widespread, though most prevalent in northern Britain, but losses can be prevented by the use of good resistant varieties of oats. *Aphelenchoides ritzemabosi* on chrysanthemums, always a serious potential pest, is now controlled by parathion dipping of stools and cuttings at a very low cost.

Dr. J. B. Goodey (Rothamsted) chose as his topic "Taxonomic Relatedness", and claimed that the recognition of new species has led to progress in solving some refractory problems in nematology. He illustrated this by referring to the fragmentation of the old taxa *Heterodera schachtii* and *H. marioni* into a number of recognizable species of *Heterodera* and *Meloidogyne* respectively. In *Ditylenchus* less progress has been made, for although the stem nematodes are now split into three species, *D. dipsaci* is still recognized as containing numerous 'biological races'. Work on *Xiphinema diversicaudatum*, a virus vector, shows that the standard monograph, containing the description of this species, appears to err. The variation shown by this nematode is extensive enough to suggest two possible species if only the extremes are taken, indicating the need to treat taxonomy dynamically with the study of plenty of living material. It was suggested that the taxonomist should not regard himself or others as infallible. The adequate description of new species requires the consideration of about 150 aspects of a nematode, and while to others his work might appear intuitive and subjective, his feet are kept firmly on the earth by the necessity to explain unambiguously his conclusions to sceptical colleagues.

In discussing "The Ecology of Soil Migratory Nematodes", Dr. R. D. Winslow (Rothamsted) defined these nematodes as those root-feeding members of the Tylenchida and Dorylaimoidea in which the female does not become cystic. In the soils investigated by him at Rothamsted and elsewhere *Pratylenchus*, *Paratylenchus*, *Tylenchorhynchus*, *Rotylenchus* and *Helicotylenchus* are most common, with *Pratylenchoides*, *Criconemoides*, *Hemicycliophora*, *Trophurus*, *Longidorus*, *Xiphinema* and *Trichodorus* occurring occasionally. In arable crops at Rothamsted and Woburn (Beds.) highest numbers of total nematodes and of total Tylenchida occur in late summer or autumn. Generally at Rothamsted, and in some cases at Woburn, these numbers are well maintained during winter, falling in spring to a minimum in May or June. Other factors greatly affecting nematode numbers are vegetation, soil-type and *p*H. Cereals generally support more plant-parasitic and other nematodes than do potatoes and sugar beet. Spiral nematodes are numerous in the heavy soil at Rothamsted; very rare in the lighter Woburn soil. *Pratylenchus pratensis* is associated with low *p*H on a hay-field at Rothamsted (probably due to *p*H affecting the botanical composition of the sward), whereas the fungivorous *Aphelenchus avenae* is associated with high *p*H. In forest nurseries *Hoplolaimus uniformis*, *Trichodorus pachydermus* and *A. avenae* are associated with relatively high *p*H.

Mr. F. G. W. Jones (Rothamsted) first briefly summed up the present state of knowledge of "The Orientation and Host-finding by Soil-inhabiting Plant-parasitic Nematodes" by saying that there appears to be no evidence of nematode response to *p*H gradients, and that tests on response to temperature gradients have not been made. There is evidence of response to moisture gradients, to carbon dioxide, to certain acidic oxidizing and reducing compounds, to root exudations of unknown constitution and possibly to the products of bacterial activity in the rhizosphere. Response to redox potentials and to electric currents is also known. He then described some of his own work on the behaviour of various nematodes, *Turbatrix aceti*, *Heterodera schachtii* larvæ and *Ditylenchus dipsaci*, when exposed to electrical gradients. Nematodes respond to electrical gradients of more than 30 mV./mm., and, as many nematodes are about 1 mm. long, this represents a change of potential from head to tail. In a gradient of 100–200 mV./mm., *D. dipsaci*, which have been moving at random, tend to move more spasmodically and to swing their heads in a wide arc. Often, after this preliminary swinging movement, the head tends to face the cathode, followed by free swimming towards it. When the current is reversed, movement follows, leading to a reversal of the direction of swimming.

Mr. C. C. Doncaster (Rothamsted) showed a film of "Some Nematode Feeding Mechanisms". This illustrated the feeding of spear-bearing nematodes and those with open stomas. In the first group, *Aphelenchus avenae* thrusts the spear repeatedly against a fungal hypha followed by a rapid pumping action of the valve of the median bulb, thus ingesting the contents of the hypha ; but in *Aphelenchoides ritzemabosi*, feeding on mesophyll cells of *Stellaria media* in a water mount, the median bulb shows only gentle pulsation while the spear is inside the cell. It was suggested that feeding in *A. ritzemabosi* may be more the tapping of cell sap under pressure. In the second group, *Diplogaster* sp., *Pelodera lambdiensis* and *Rhabditis oxycerca* are saprophages. In *Diplogaster*, food particles are sucked in by the expansion of the tri-radiate lumen of the corpus (first bulb), which acts rather like a pipette. The lumen through the base of the corpus can be closed completely and acts as a valve. From the first bulb, food is transferred through the isthmus and second bulb by a wave of peristaltic-like action caused by successive contractions of the radial muscles surrounding the tri-radiate lumen of both parts. In *P. lambdiensis* and *R. oxycerca* the second bulb has three equally spaced valve plates which move backwards into a cup-like cavity. The whole process of feeding is very complex, but appears to be divisible into the following stages: (*a*) the procorpus and corpus open, drawing in food and, on closing, food collects in the forepart of the isthmus; (*b*) this food mass is carried further back to the valve by a wave of 'peristalsis'; (*c*) the cup behind the valve dilates, sucking the food through the valve flaps, which then flick back into the cup together with the whole of the anterior part of the valve; (*d*) the cup collapses, forcing the food into the œsophago-intestinal canal behind it, this canal also showing 'peristaltic' dilation and collapse.

Dr. H. R. Wallace (Rothamsted), in discussing "Plant Reaction to Parasitism by Nematodes", listed some of these reactions; he mentioned rounding of cells and loosening from one another, possibly by the action of pectic enzymes in tissues attacked by *Ditylenchus dipsaci*; the formation of giant cells in roots attacked by *Heterodera* spp. and *Meloidogyne* spp., possibly indicating the presence of growth hormones; cell hyperplasia and hypertrophy in roots attacked by *Radopholus similis*, and extensive necrosis associated with the invasion of roots by *Pratylenchus* spp. He went on to describe his work on the attack in chrysanthemum leaves by *Aphelenchoides ritzemabosi*. In susceptible varieties few cells appear to be attacked, the nematodes remain very near their point of entry, and reproduction goes on; but in resistant varieties many cells are attacked, the nematodes move far and wide and there is almost no reproduction. Resistant varieties show rapid and extensive leaf discoloration associated with the enzymic oxidation of plant polyphenols, and it is suggested that there is an absence of some nutritional factor in these leaves. Dr. Wallace ended by stressing the state of our ignorance of the inter-relationships, particularly biochemical ones, of nematode parasite and plant.

Dr. R. S. Pitcher (East Malling Research Station) spoke on "Nematode Vectors of Virus Diseases", and said that it was the pioneer work of Hewitt and Raski in California that first showed the transmission of virus by nematodes. He stressed that though this work is important in its own right, it should also stimulate study of the complex inter-relationships of nematodes and other 'soil inhabiting' pathogens and their relation to the plant and its environment. Of the 'soil-borne' viruses, those that have been transmitted by nematodes could be classified as having infectivity destroyed by air-drying of soil, and while 'tobacco rattle', which is transmitted by *Trichodorus pachydermus*, has rod-shaped particles, the others have isometric particles, for example, 'fanleaf of grape', 'peach yellow bud mosaic' and 'arabis mosaic' transmitted by *Xiphinema index*, *X. americanum* and *X diversicaudatum* respectively and 'tomato black ring' transmitted by *Longidorus elongatus*. There is a clear correlation of disease and the presence of nematodes which are all members of the Dorylaimoidea, with relatively long spears when compared with most members of the Tylenchida, none of which is known as a virus vector. The means by which virus transmission takes place is unknown and, as yet, a matter for speculation.

"Practical Problems and Recent Trends in Nematode Control" was the topic chosen by Mr. F. C Peacock (Plant Protection, Ltd., Jealott's Hill), who said that many of the earliest nematicides which still give good service are halogenated hydrocarbons. They are generally phytotoxic and relatively volatile substances. Methyl bromide and D–D are early examples and 1,2-dibromo-3-chloropropane a more recent one. A second group of nematicides are the organic phosphates: parathion has been used for some years; more recent examples are O-2,4-dichlorophenyl-, O-diethyl phosphorothioate and diethyl pyrazinyl phosphorothioate. This group of compounds is non-volatile and relatively insoluble in water, but has high nematicidal and residual value and low phytotoxicity, albeit high mammalian toxicity. Dithiocarbamates make good soil sterilants but have high phytotoxicity; their action against nematodes is said to be due to the breakdown product methyl *iso*thiocyanate. Various sulphur compounds, such as chlorophenyl methyl rhodanine, have high phytotoxicity which is useful in seed-bed preparation, but the tetrahydrothiophene dioxides and trithiophene (occurring in *Tagetes erecta*) have low phytotoxicity. Recently the use of yellow oxide of mercury incorporated in soil has shown promise. The observation that a glycoside from *Asparagus officinalis* roots inhibits nematode attack has led to an investigation of the systemic use of maleic hydrazide and other substances, applied to leaves to prevent attack on roots either by the exudation of toxic material or the inhibition of attractive diffusates, or theoretically the preferable killing of already established parasitic nematodes. Mr. Peacock finished by stressing the need for good husbandry so that nematicides are only supplementary in function, and the need for recognizing that different nematode diseases need treatment by different nematicides.

J. B. GOODEY

AREA OF RESIDENCE OF MENTAL HOSPITAL PATIENTS

ONE of the Registrar General's series of studies on medical and population subjects contains detailed statistics about the areas from which patients were admitted to mental hospitals in England and Wales during 1957. (*Studies on Medical and Population Subjects*, No. 16; "Area of Residence of Mental Hospital Patients", H.M.S.O. 15*s*. 6*d*. net.) The addresses of patients at the time of admission have been classified according to the administrative areas in which they are situated. Separate tables are given showing, for the first time, the numbers of admissions from each county borough and administrative county and from each metropolitan borough in the County of London. Figures are given separately for each sex and for certain age-groups and diagnoses.

Total admissions to mental hospitals during 1957 numbered 88,943, of which 48,266 (or 54 per cent) related to patients admitted to a mental hospital for the first time. Separate figures are given for total admissions (that is, first admissions and all re-admissions) and first admissions from each area and comparisons can be made on either basis. Most of the figures which follow are based on the total number of admissions and not on the number of persons admitted and do not therefore necessarily reflect differences in the proportion of persons in an area who were admitted to a mental hospital during the year.

Of the total number of admissions, 6,859 came from the County of London, while admissions from the counties of Middlesex, Kent, Essex, Surrey and Hertfordshire numbered 4,722, 3,674, 4,054, 3,214 and 1,571 respectively, giving a total of 24,094 for London and the surrounding counties. Among the counties outside Greater London, the highest numbers of admissions were of residents in Lancashire (2,947), and the West Riding of Yorkshire (2,395).

Although the volume only contains figures of the numbers of admissions, it is possible to relate these figures to the estimated populations of the areas at June 30, 1957, to give admission-rates per 1,000 population. The ten county boroughs with the highest number of admissions, with the corresponding admission-rates per 1,000 of the resident population, were as follows :

Birmingham	2,096 (1 9)	Sheffield	812 (1·6)
Leeds	1,023 (2·0)	Cardiff	761 (3·0)
Liverpool	1,005 (1·3)	Stoke-on-Trent	735 (2·7)
Manchester	836 (1·2)	Kingston upon Hull	702 (2·3)
Bristol	823 (1 9)	Bradford	622 (2 2)

Admission-rates for Lancashire and the West Riding of Yorkshire were 1·4 and 1·5 per 1,000 respectively.

Among individual metropolitan boroughs of London those having the highest rates of admission were Stoke Newington with 2 19 (144 admissions) per 1,000 and Stepney and Chelsea both with 2 8 per 1,000 of the population (274 and 144 admissions respectively).

The higher rates of admission are not concentrated in those counties with a greater degree of industrialization. For example, the rates for Staffordshire and Durham were 1·7 and 1·6 compared with 2·5 and 2·1 per 1,000 of the population, respectively, for Cornwall and Norfolk.

There were 1,553 admissions of patients stated to have no fixed abode, or 1·7 per cent of the total number admitted. Among these the number of re-admissions was 913 or 59 per cent, a figure well above the national percentage of 46. The admissions of patients normally resident outside England and Wales numbered 188 or 0 2 per cent of the total number of admissions.

Among the causes of admission, manic-depressive reaction contributed the largest proportion for England and Wales as a whole. Of the national total of 4,868 admissions due to character, behaviour and intelligence disorders, 440 were from the County of London and a further 322 were of people with no fixed abode. In London, 1,954 out of the 6,859 admissions resulted from schizophrenia (28 per cent) and 30 per cent were for manic-depressive reaction.

The proportions due to schizophrenia among the admissions from the ten county boroughs shown above were :

Birmingham	22 per cent	Sheffield	21 per cent
Leeds	14 ,, ,,	Cardiff	18 ,, ,,
Liverpool	31 ,, ,,	Stoke-on-Trent	22 ,, ,,
Manchester	36 ,, ,,	Kingston upon Hull	24 ,, ,,
Bristol	20 ,, ,,	Bradford	18 ,, ,,

By comparison, the proportions due to schizophrenia among the admissions from the predominantly rural counties of Cornwall and Herefordshire were 11 and 9 per cent respectively.

Of the first admissions for the whole country, 6,906 were reported as due to schizophrenia (14 per cent) compared with 13,902 admissions or 29 per cent for manic-depressive reaction.

COMMUNITY MENTAL HEALTH

With the aid of a generous grant from the Nuffield Provincial Hospitals Trust, Political and Economic Planning has recently begun a three-year study of the mental health services of Britain with particular emphasis on the subject of community care. The work is under the control of a steering group, the director of research being Dr. F. M. Martin, of the University of Edinburgh. A broadsheet, entitled "Community Mental Health Services", gives a general background to the work and shows why, and in what directions, research is necessary*.

The greater part of the research programme of Political and Economic Planning will take the form of a study of the actual operation of community mental health services in selected areas. Districts will be chosen for study primarily because they have already developed distinctive and interesting patterns of community care, such as might prove models for other areas. It is hoped that, in at least some areas, it will be possible to work in association with locally based research workers who are already interested or actively concerned in the study of mental health services. An assessment of a particular form of organization of services must take account of the statistics of patient movement, but these cannot be adequately evaluated in isolation. It will be particularly important to make some estimate of the social and personal consequences for patients and their families, of different patterns of care, and to study closely the nature of the community services provided.

In view of the wide variety of workers likely to be engaged in community care, and of the contradictory views which have been expressed on their place in the mental health services, it would be of interest to attempt some systematic analysis of the work actually performed by health visitors, psychiatric social workers, mental welfare officers, and so on. In this way it should be possible to set an analysis of professional functions against an analysis of the needs of patients and families, and to consider how effectively they can be related to one another. A comprehensive area study should also take account of the part played by voluntary organizations and of the work of the family doctor, whose potential short-term and long-term contribution to a community-oriented mental health service has yet to be satisfactorily defined.

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* *Planning*, 26, No. 447 (12 December, 1960) · "Community Mental Health Services". Pp. 337–356. (London: Political and Economic Planning, 1960.) 2*s*. 6*d*.

In the mental deficiency field, where there is a longer tradition of community care, there seems to be fewer self-conscious experiments in progress outside the hospital service. Most local authorities appear to believe that the desirable pattern of care has been fairly well worked out, and that they know at what goals they should be aiming. There is little or nothing on the mental subnormality side comparable to the research now in progress, or being planned, for the mentally ill. There may therefore be fewer opportunities for collaborative research, but it would be desirable, in areas selected for intensive study, to examine how mental subnormality services function, and how far they meet the needs of the mentally subnormal and their families.

Another issue, deeply involved in the functioning of mental health services, is that of public attitudes towards mental disorders. The beliefs and attitudes which are, in fact, prevalent in different segments of society must inevitably influence the way in which mental health services function. They are likely to affect what is defined and recognized as 'mental illness,' to condition the response of families to illness in one of their members, and to influence both the demand for treatment and the post-hospital resettlement of former patients. There have not been many systematic studies of attitudes towards mental disorder; but it seems that the structure of such attitudes is, in fact, complex, that their psychological roots are deep, and that they have an important irrational component. It may be profitable to consider undertaking certain limited studies of attitudes and beliefs, possibly in the areas chosen for general investigation. The studies may be confined, in the first instance, to the close relatives of psychiatric patients, and to certain professional groups having special responsibilities for the care of the mentally disordered.

The scope of Political and Economic Planning's inquiry will depend on the degree of collaboration with other workers, and on the extent to which further resources may become available. There is every reason to hope that even a modest set of operational studies of the work of the mental health services may make a useful contribution to the further development of community care.

THE HYDROCHARITACEÆ IN NEW ZEALAND

ONE member of the tribe Anachriteæ of the family Hydrocharitaceæ, *Elodea canadensis*, has long been established in New Zealand and is now well known and widespread. R. Mason, of the Botany Division, Department of Scientific and Industrial Research, Christchurch, has now collated information from recent expeditions about this and other species in the family (*New Zealand J. Sci.*, 3, No. 3; September 1960).

E. canadensis has not been as aggressive or so fast-spreading in New Zealand as in Britain, in spite of its initially rapid spread in New Zealand. Cheeseman (1920), when recording it for Featherston, said, "Considering how rapidly this plant increased when first introduced into Britain, it is somewhat remarkable that its spread in New Zealand has been slow since its first introduction in 1870". In 1906, 38 years after its introduction, Cheeseman had only three listed districts, Mongonui, the Bay of Plenty, and the Canterbury Plains. In 1922 Thomson said it "is now abundant throughout New Zealand". "Judging by its distribution as known at present, if Cheeseman perhaps understated the extent of its distribution, Thomson overstated it". As New Zealand conditions are different from those in Britain or Europe in many ways, the behaviour of this plant would be expected to differ. In New Zealand *E. canadensis* would have met more vigorous competition from watercress than in Britain, for watercress had been introduced in New Zealand less than twenty years before and "it immediately spread aggressively and grew to an immense size far exceeding anything seen in Great Britain".

It is not known how abundant *Egeria densa* was in the Waikato when it was first collected there in 1946, but to-day it seems the commonest weed in the river from Rangiriri downstream as far as fresh water aquatics go—some 35 miles or so. In 1958, large beds of the weed were found wherever the river was examined; the weeds reached the surface along the river edges and formed submerged weed beds well into deeper waters. In Whangape Lake, it was found to form broad beds a short distance off-shore, apparently stretching all the way around the edge.

In Waihou River, where it was found in November, 1959, it seemed to be scattered in patches rather than in large-size, continuous beds.

In 1950, when *Lagarosiphon major* was found in New Zealand, it almost covered the bed of the Waiwhetu Stream in its lower reaches above tidal influences. According to A. P. Druce it is still abundant and is mixed with *Elodea canadensis*. Druce also recently found it in a cut-off of the Hutt River behind Barton's Bush, about 8½ miles from Woburn, the nearest previously known station.

In Lakes Rotorua, and in Hamilton Lake, growth of this weed is sufficiently great to interfere seriously with fishing, boating and swimming. At Rotorua the position has become so alarming that a special committee, including representatives of the Departments of Agriculture, Lands and Survey, Internal Affairs and the Marine Department, has been formed to tackle the problem of controlling and destroying the weed.

The weed has only recently begun to grow with such vigours, although it has been present in the Lake for a long time. It has been suggested that aerial top dressing in the district has suddenly increased the fertility of the Lake water; it is far more likely that the less-vigorous *E. canadensis* has been present for many years and that *L. major* is a much more vigorous newcomer attracting attention.

Mason also describes the establishment and means of distribution of all three species.

CHOICE OF TREE SPECIES

A PUBLICATION on forestry issued by the Food and Agriculture Organization tends to make one ask: "What has forestry to do with food and agriculture?" In the introduction to a recent Forestry Development paper entitled *Choice of Tree Species**, we are told that it is a contribution to the general *World Forest Planting Manual*, about which it gives no more information. One next asks: For whom is this work intended? Its primary object is stated to be to summarize what is known about the factors which should guide the practising forester in reaching a decision about the choice of species. The second object is said to be to stimulate foresters to amplify and correct the often inadequate information presented in this book. With regard to the first object it must be said right away that the summary is extremely faulty in respect both of the completeness of the information and of the manner of its treatment. With regard to the second object, this seems to imply that the aim of a publication is not to furnish information but to ask for information.

This type of publication is a feature of the present day. The setting up and staffing—at considerable expense—of global organizations from above downwards instead of building them on well-founded services from below upwards lead to attempts being made to produce text-books intended to be of universal use. The time has not yet come when natural agencies will have reduced the face of the globe to one monotonous landscape. Perhaps in no other field is there a greater variety of working conditions than in forestry. As the author, or authors, say (p. 69), "It is, of course, quite impossible to suggest general rules on the selection of species and sites for production which would be applicable in all circumstances". Nevertheless, they have attempted the impossible. Then again (p. 170) it is admitted that "The information available from different parts of the globe is so variable that uniform presentation is not possible". Uniform presentation has not been achieved. There are very serious gaps in the sources consulted, especially in respect of European countries; some works in English on the subject have been overlooked; recent developments in the study of the major site factors are unrecorded; conclusions are accepted and illustrations reproduced from examples of plantations too young to have proved their suitability; there is a lack of discrimination in assessing the relative importance of the many publications quoted; there is a very evident bias in the selection of material and too narrow an outlook on this extremely difficult problem.

The fact is that it would be unwise to recommend to any practising forester that he should purchase and consult this book—or any book of such a global type; it would be far better that he should be advised to consult the literature available in respect of the narrower field with which he is concerned, or of areas like it. To help him in this, all that is required is information on the literature referring to his conditions, to furnish which is the task—admirably performed—of the Commonwealth Forestry Bureau, Oxford.

The age of the superman has not yet arrived. No one is qualified to undertake satisfactorily such a tremendous task as that attempted in this case. The eyes of a fool are in the ends of the Earth. It was unwise of the Food and Agriculture Organization to sponsor this book, and one can only offer sympathy to those who were enticed into wading through a vast amount of heterogeneous literature to produce it. It is understood that the Organization is assisting with forestry publications dealing with certain restricted geographical regions. This is more rational and should prove more useful.

If one is eclectic and chooses the parts of special interest to oneself, there is something of value to be had from the book. Part 3, for example, consists of monographs on four widely differing species—Norway spruce, Monterey pine, teak and blue gum, which demonstrate effectively the wide differences among tree species which render difficult the writing of a global text-book. M. L. ANDERSON

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* Food and Agriculture Organization of the United Nations. FAO Forestry Development Paper No. 13: *Choice of Tree Species*. Pp. xi+307. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1960.) 17*s*. 6*d*.; 3.50 dollars.

ENERGY-LINKED CYTOCHROME OXIDATION IN MITOCHONDRIA

By PROF. BRITTON CHANCE

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THE study of succinate-linked reduction of pyridine nucleotide in mitochondria was begun some time ago[1]. More recent work demonstrates that reduction of diphosphopyridine nucleotide in mitochondria is an energy-linked reaction having a definite requirement for internal high-energy intermediates[2] or for adenosine triphosphate itself[3]. Although previous attempts to demonstrate a requirement for adenosine triphosphate failed[2], these recent experiments demonstrate the possibility of driving electron transfer reactions in the respiratory chain in reverse by means of high-energy intermediates, and it has been of interest to determine whether or not cytochrome reduction induced by substrate could be reversed by adenosine triphosphate, a possibility that is particularly attractive in connexion with theories of active transport[4,5] and photosynthesis as well. (Lundegårdh[6] has reported an effect of adenosine triphosphate (and fumarate) on the interaction of cytochromes *b* and *dh* in anaerobic wheat roots, cytochrome *dh* being "turned over into a state of strong reduction under the influence of adenosine triphosphate (and fumarate) simultaneously with cytochrome *b* remaining more oxidized". The interpretation of this interesting result is rendered ambiguous by the fact that the existence of cytochrome *dh* in wheat roots and other plant or animal tissues has not been confirmed by other workers[7] nor by us using liquid-nitrogen spectroscopy of wheat root mitochondria (B. Chance and W. D. Bonner, jun., unpublished observations).)

The present article reports that addition of adenosine triphosphate causes the oxidation of cytochromes of mitochondria in the presence of dithionite or terminal respiratory inhibitors such as cyanide or sulphide. Stoichiometric and kinetic results indicate that this is a fairly efficient reaction; inhibitor studies suggest more than one site of interaction of adenosine triphosphate with the respiratory chain. The relationship of these results to the responses of cytochromes to adenosine diphosphate[8] and to the adenosine triphosphatase activities of mitochondria are discussed. It is apparent that the oxidation-reduction states of the cytochrome, flavoprotein and the pyridine nucleotide components of the respiratory chain are as well controlled by the adenosine triphosphate and adenosine diphosphate + inorganic phosphate-levels as by reductant and oxidant.

Preparations. Pigeon heart mitochondria were prepared by a procedure developed in this laboratory (Chance, B., and Hagihara, B., in *Proceedings of the Fifth International Symposium of Biochemistry*, in preparation) involving the use of *B. subtilis* proteinase. With succinate as substrate such mitochondria have respiratory control ratios >3 (usually 4–5) and P/O values of 1·8 with respiratory activities corresponding to a turnover number of cytochrome *c* of 6 sec.$^{-1}$ at 26°. Freshly prepared rat liver mitochondria[9] and 'digitonin particles'[10] were also used. (Thanks are due to Mr. K. Kaminker and Miss H. Diefenbach for pigeon-heart preparations and to Dr. U. Fugmann for 'digitonin' preparations.) The reaction medium was free of phosphate and magnesium and consisted of 0·27 *M* mannitol, 0·03 *M* sucrose and 0·01 *M* *tris* *p*H = 7·3. The temperature of the experiments was 26°. The cytochrome *c* concentration under the conditions of the experiment ranged from 1 to 2 μ*M*.

The double-beam spectrophotometer[11] set at appropriate pairs of wave-lengths for cytochrome components was used simultaneously with a recording fluorometer for measuring pyridine nucleotide reduction. The latter employed 365 mμ for excitation and 450 mμ for recording[12], these wave-lengths being obtained by appropriate filters.

The split-beam spectrophotometer was used at room and liquid-nitrogen temperatures to identify cytochromes affected by the addition of adenosine triphosphate. The optical path-length was 1 mm. at low temperatures. The 'trapped steady state' method[13] was used.

Cytochrome Oxidation in the Presence of Sodium Dithionite

Although cytochrome oxidation is observed on addition of adenosine triphosphate in the presence of terminal respiratory inhibitors, there is the remote possibility in such experiments that adenosine triphosphate might somehow have interfered with the action of the inhibitor and have induced a cytochrome oxidation that was linked with oxygen and not with adenosine triphosphate. In experiments with dithionite, controls were carried out to indicate that an excess of dithionite was added so that the subsequent addition of adenosine triphosphate to the solution did not stir in sufficient oxygen to exhaust the added dithionite. This was done by titrating the dithionite solution with oxygen by means of the platinum microelectrode.

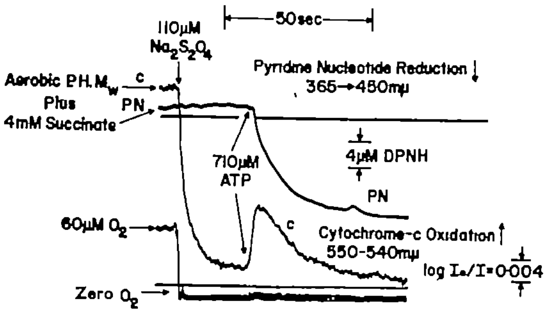

Fig. 1. Oxidation of cytochrome *c* and reduction of pyridine nucleotide caused by the addition of adenosine triphosphate to anaerobic pigeon heart mitochondria treated with dithionite, suspended in mannitol–sucrose–*tris* medium (1·9 mgm. protein/ml.). Concentrations of reactants added and the absorbancy and the concentration scales are indicated on diagram (Exp. 193*b*).

An experiment typical of this work is indicated in Fig. 1. Aerobic pigeon-heart mitochondria (essentially free of endogenous substrate) are treated with 4 m*M* succinate and cytochrome *c* is recorded spectrophotometrically, an upward deflexion of the trace corresponding to an oxidation. Pyridine nucleotide reduction is measured fluorometrically; a downward deflexion of the trace corresponding to increased fluorescence and increased reduction of pyridine nucleotide. The initial oxygen concentration of the suspension was diminished by bubbling nitrogen through the reaction medium, so that a value of 60 instead of 240 μ*M* is obtained. The dithionite solution calibrated as described above is added to a final concentration of 110 μ*M* in oxygen equivalents and causes an abrupt decrease of the oxygen concentration to zero, as indicated by the fall of the platinum electrode trace. At this time there is a rapid reduction of cytochrome *c*. There is no reduction of diphosphopyridine nucleotide, verifying that these mitochondria are free of substrate capable of directly activating reduction of this substance. As soon as the anaerobic steady state is obtained, an excess of adenosine triphosphate is added (710 μ*M*), and there is an abrupt oxidation of cytochrome *c* and a reduction of pyridine nucleotide, the rates being respectively 0·42 μ*M* iron/sec. and 1·2 μ*M* reduced diphosphopyridine nucleotide/sec. The oxidation of cytochrome *c* is transitory and its reduction soon follows, the half-time for the cycle of oxidation and reduction of cytochrome *c* being about 15 sec. The reduction of pyridine nucleotide proceeds exponentially to completion, and no re-oxidation of this component is observed. This experiment indicates that the addition of adenosine triphosphate to anaerobic mitochondria causes an abrupt cyclic oxidation of cytochrome *c* and a simultaneous reduction of diphosphopyridine nucleotide.

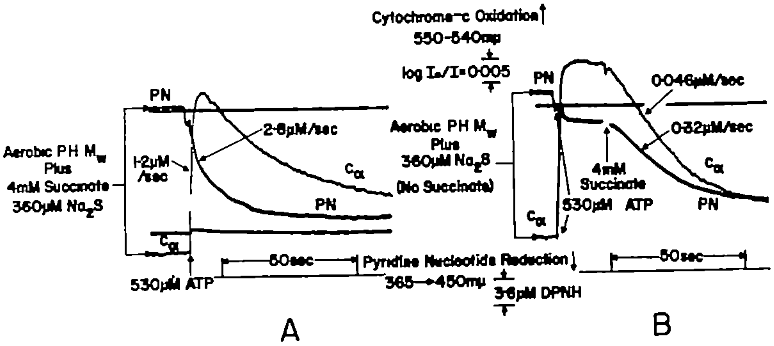

Fig. 2. Oxidation of cytochrome *c* and reduction of pyridine nucleotide in pigeon heart mitochondria inhibited with 360 *μM* sulphide. Left, in the presence of succinate and, right, in the absence of succinate. Concentrations, absorbancy calibrations and time scales are included in the figure. Mannitol–sucrose–*tris* medium, 1·1 mgm. protein/ml. Temperature, 26° (Exp. 185*b*-3)

Cytochrome Oxidation in the Presence of Terminal Respiration Inhibitors

Results similar to those of Fig. 1 have been obtained in the presence of excess cyanide or sulphide. An example of the latter is illustrated in Fig. 2*A*. In this case the mitochondria are pretreated with 4 m*M* succinate and 360 *μM* sodium sulphide. This causes complete reduction of cytochrome *c* but negligible reduction of pyridine nucleotide. On the addition of 530 *μM* adenosine triphosphate, there is an abrupt oxidation of cytochrome *c* at a rate of 1·2 *μM* iron/sec. and a corresponding reduction of diphosphopyridine nucleotide at a rate of 2·8 *μM*/sec. The maximal rate of oxygen utilization in the absence of sulphide and in the presence of phosphate + adenosine diphosphate (state 3) is 4·8 *μM*/sec. in 2-equivalent units. Thus the rate of electron transfer elicited by addition of adenosine triphosphate is high. Similar results are obtained with rat liver and brain mitochondria and with 'digitonin particles'.

As in the case of the mitochondria treated with dithionite, the cytochrome oxidation is cyclic, although longer-lived. Further additions of adenosine triphosphate cause smaller cyclic responses of the cytochromes.

Substrate Requirements for Pyridine Nucleotide Reduction and Cytochrome Oxidation

In Fig. 2*B* the experiment shown in Fig. 2*A* is repeated without succinate. There is a slow reduction of cytochrome *c* on addition of sulphide due to traces of endogenous substrate. Here the addition of adenosine triphosphate causes an abrupt oxidation of cytochrome *c* to a higher level than was obtained in the presence of succinate. Pyridine nucleotide shows only a small reduction compared with that obtained in material treated with succinate; only 20 per cent is reduced. Addition of succinate to the material treated with adenosine triphosphate allows reduction of pyridine nucleotide to proceed to the same extent that was observed in Fig. 2*A*. At the same time, cytochrome *c* reduction proceeds, but at a slower rate than the rate of pyridine nucleotide reduction. This record confirms the previous results, suggesting a specific requirement for succinate in energy-linked reduction of diphosphopyridine nucleotide[8].

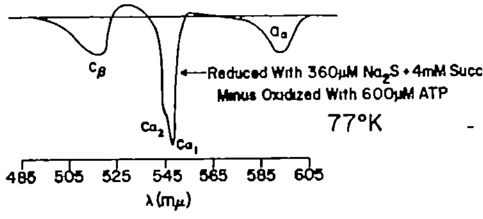

Fig. 3. Low-temperature 'trapped steady state' of oxidation of cytochromes *c* and *a*. Pigeon heart mitochondria treated as indicated in the figure, 1·2 mgm. protein/ml. Optical path 1 mm. (Exp. 185*b*–6)

Effect upon Other Cytochromes

To determine that a cytochrome oxidation has definitely occurred and to identify other cytochromes affected by addition of adenosine triphosphate, the 'trapped steady state' method of low-temperature spectroscopic observations has been employed in which the material in both cuvettes of the split-beam recording spectrophotometer is pre-treated with 360 *μM* sodium sulphide and 4 m*M* succinate. Just prior to freezing, 600 *μM* adenosine triphosphate is added to one of the cuvettes, and the spectrum representing the difference in absorbancy between the two is plotted in Fig. 3. In the interval (about 15 sec.) between addition of adenosine triphosphate and freezing, absorption peaks corresponding to cytochromes *c* and *a* have disappeared, the α_1 and α_2 peaks of the former being clearly identified. No indication of cytochromes c_1 or *b* is given in this record, and it may be presumed that directly on addition of adenosine triphosphate their oxidation states have not been measurably altered.

Time-Course of Cytochrome Change

To determine the time-sequence of the oxidation and reduction of cytochromes *c* and *a*, and to determ-

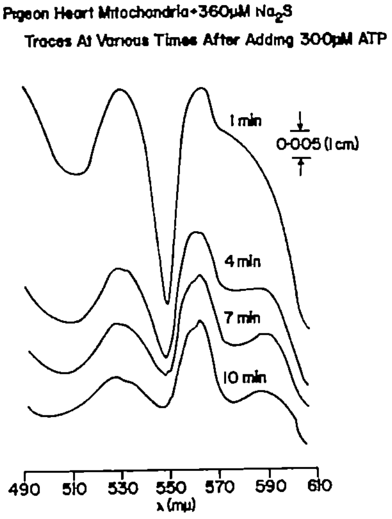

Fig. 4. Room temperature difference spectra showing the time course of the changes in oxidation- and reduction-levels of cytochromes *a*, *c* and *b*. Experimental conditions similar to those of Fig 2*B*, 1 cm. optical path (Exp. 185*c*–3)

ine whether or not any slow effects on cytochrome *b* can be observed, we have recorded (Fig. 4) in the split-beam spectrophotometer room temperature difference spectra at three-minute intervals after adding 300 μM adenosine triphosphate to mitochondria treated with 360 μM sodium sulphide (succinate being omitted, cf. Fig. 2*B*). In accordance with the results of Fig. 3, the record shows clearly the oxidation of cytochromes *c* and *a* as indicated by the disappearance of absorption peaks at 550 mμ and at 605 mμ. Since this record was traced at about 1 min. after adding adenosine triphosphate, there already appears a distinctive peak at approximately 560 mμ. After a 4-min. wait, it is observed that the oxidation of cytochrome *c* is considerably smaller, as is that of cytochrome *a*. In fact, at the end of the fourth recording, the oxidation of cytochromes *c* and *a* has subsided almost completely. The spectroscopic effect in the region of cytochrome *b* has developed and a distinct peak indicating reduction of cytochrome *b* has appeared. This peak is broad and suggests the oxidation of more than one component.

Stoichiometry of Adenosine Triphosphate-Cytochrome Reaction

In view of the considerable interest in adenosine triphosphate-driven production of electron transfer for the purposes of active transport, the stoichiometry between adenosine triphosphate and cytochrome oxidation is relevant. Fig. 5*A* illustrates the titration of cytochrome *c* oxidation with adenosine triphosphate in the absence of succinate, and it is seen that a plateau is reached with less than 10 μM adenosine triphosphate. The titration curve is sigmoid, as if some other substances were being titrated prior to cytochrome *c*, or as if a certain redox potential were required. However, a stoichiometric relationship for adenosine triphosphate and cytochrome *c* is taken from the steepest part of the curve, which corresponds to 3 molecules of adenosine triphosphate per cytochrome *c*. Since cytochrome *c* is only one of four components observed to be oxidized simultaneously, the adenosine triphosphate/electron value is approximately 1. This value corresponds fairly well with the value of adenosine triphosphate/reduced diphosphopyridine nucleotide of 1·8–2·7 for reduction of diphosphopyridine nucleotide in mitochondria treated with succinate[3]. If the total adenosine triphosphate added is divided by the total electron equivalents found in cytochromes *c*, *a* and a_3 and flavoprotein, then the ratio is larger ; 5·6 μM adenosine triphosphate gave a total of only 1 5 one-electron equivalents oxidized, presumably due to the sigmoid nature of the titration (Fig. 5*A*).

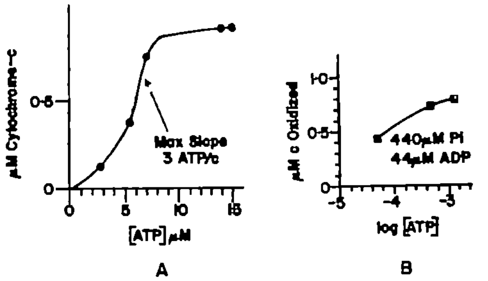

Fig. 5. Titrations of cytochrome *c* of mitochondria treated with sulphide (no succinate added) under conditions to determine an adenosine triphosphate/cytochrome 'stoichiometry' (part *A*) and an 'equilibrium' (part *B*). In the latter case phosphate and adenosine diphosphate are present. The total cytochrome concentration ($c + c_1$) was 1 2 μM in part *A*, 1·8 μM in part *B*; 360 μM sodium sulphide, 26°, mannitol–sucrose–*tris*-medium, pH = 7·4 (Exps. 198*b*, 203)

Nucleotide Specificity of the Reaction

In order to determine whether there is absolute specificity for adenosine triphosphate, we have tested the effect of various nucleotides on pigeon heart mitochondria that have been treated with succinate and sulphide as in the previous experiments. The addition of the 5′-triphosphates of ribosyl guanine, uracil, hypoxanthine and cystosine (72 μM) gives no response (less than 1 per cent), whereas adenosine triphosphate causes changes observed in Fig. 2*A*. This result is in accordance with the specificity of oxidative phosphorylation for adenosine diphosphate[14], but differs from the specificity of adenosine triphosphatase stimulated by dinitrophenol and magnesium[15].

Effects of Adenosine Diphosphate, Inorganic Phosphate and Magnesium, etc.

Experiments similar to those of Fig. 2*A* show that both adenosine diphosphate and phosphate inhibit the rate and extent of the reaction, adenosine diphosphate being considerably more effective than phosphate—an expression of their relative affinities in oxidative phosphorylation. A combination of adenosine diphosphate and phosphate is much more inhibitory, as would be expected from equation 1 (see later). It is possible to titrate cytochrome *c* with adenosine triphosphate in the presence of adenosine diphosphate and inorganic phosphate under the conditions of Fig. 2*B* (no substrate added) and an experiment on this point is given in Fig. 5*B*. It is seen that 50 per cent oxidation of cytochrome *c* can be obtained with a ratio [ADP] [Pi]/[ATP] of roughly 100, suggesting that in the absence of added substrate, oxidation of cytochrome *c* is not difficult to 'drive'. Magnesium is inhibitory and 0·7 mM magnesium largely blocks the effects of 32 μM adenosine triphosphate under conditions similar to those of Fig. 2*B*, presumably by activation of an adenosine triphosphatase that competes with the cytochrome reaction.

Dinitrophenol (4·5 μM) inhibits cytochrome-c oxidation by 50 per cent. Oligomycin (1 gm./ml.) inhibits completely. Thus the energy transfer pathway by which ATP activates reversed electron transfer is identical to that by which ADP is phosphorylated to ATP.

Effect of Ageing

Ageing of the mitochondria to the extent that respiratory control is impaired causes a considerable inhibition of the rate and extent of pyridine nucleotide reduction and of cytochrome oxidation. The cycle of oxidation and reduction of cytochrome *c* caused by the addition of adenosine triphosphate is considerably shorter, suggesting that the latter is expended not only in an energy-conserving reaction of diphosphopyridine nucleotide reduction and cytochrome oxidation, but also in the dissipative reaction involving hydrolysis of adenosine triphosphate.

Correlation with Adenosine Triphosphatase Activity

Simultaneous measurements of breakdown of adenosine triphosphate upon its addition to mitochondria treated with succinate and sulphide have been made by pH measurement[16] (unpublished work in collaboration with Drs. Saris and Ito) in order to determine the time relationships of adenosine triphosphate breakdown and the cytochrome and pyridine nucleotide kinetics. It has been found that the breakdown of adenosine triphosphate under these conditions begins at the same time as the oxidation of cytochrome and the reduction of pyridine nucleotide. It is further observed that the molar rate of breakdown of adenosine triphosphate somewhat exceeds the maximal rate of reduction of pyridine nucleotide. The rates of breakdown of adenosine triphosphate under these conditions have been compared with those observed in the dinitrophenol-activated breakdown of adenosine triphosphate and are found to be one-half to one-third the maximal rate observed. These results do not identify the pathway of breakdown of adenosine triphosphate in adenosine triphosphatase activity with the pathway of adenosine triphosphate utilization in cytochrome oxidation at the present time.

Effects of Inhibitors of Electron Transfer

Since adenosine triphosphate can interact with pairs of respiratory enzymes and does not require electron transfer through the whole chain, the effects of inhibitors on reversed electron transfer differ from those on oxidative phosphorylation obtained by electron transfer through the complete chain. Amytal, quinoline-N-oxide, and antimycin *A* slow the responses of the carriers to adenosine triphosphate, but do not eliminate slow cyclic responses of cytochromes *a* and *c*. Flavoprotein and reduced diphosphopyridine nucleotide respond to the initial addition of adenosine triphosphate (see Fig. 6), but after several further additions of the latter, the response of these components becomes negligible, pyridine nucleotide being completely reduced. In the system blocked with quinoline-N-oxide or antimycin *A*, the rate of cytochrome oxidation is so slow that electron transfer through the inhibited site to an electron acceptor such as ubiquinone is a possibility.

The oxidation of flavoprotein and the reduction of pyridine nucleotide in the system blocked with quinoline-N-oxide would appear to indicate an interaction site for adenosine triphosphate between flavoprotein and pyridine nucleotide.

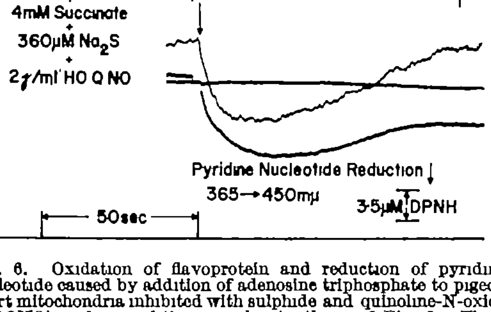

Fig. 6. Oxidation of flavoprotein and reduction of pyridine nucleotide caused by addition of adenosine triphosphate to pigeon heart mitochondria inhibited with sulphide and quinoline-N-oxide (HOQNO) under conditions similar to those of Fig. 2. Time, absorbancy, and concentration scales indicated on figure, 1·2 mgm. protein/ml. (Exp. 178)

Discussion : Relation to Active Transport and Photosynthesis

The results presented here indicate that specific oxidation-reduction reactions are induced by the addition of adenosine triphosphate to mitochondria devoid of oxidase activity due to removal of oxygen by sodium dithionite or due to respiratory inhibition by sodium sulphide. This reaction constitutes a genuine conversion from adenosine triphosphate to electron transfer, a process that has been sought for a number of years. However, it was apparent that the energy-linked reduction of diphosphopyridine nucleotide[2,3] would be accompanied by an energy-linked oxidation, provided suitable experimental conditions were found. The appropriate conditions are simple ; electron transfer proceeds rapidly in the reverse direction in mitochondria that are relatively free of magnesium, adenosine diphosphate and inorganic phosphate. This discovery may be of considerable importance to our understanding of the function of the respiratory chain, and to our consideration of new ways in which metabolic processes could be affected by the transfer of the energy of adenosine triphosphate to oxidation-reduction equivalents.

It is useful to consider, on the basis of these results, the appropriate conditions for reversed electron transfer in the living cell. In intact mitochondria in anaerobiosis, a high 'phosphate potential' (see below), generated by glycolytic activity, could cause appreciable reduction of mitochondrial diphosphopyridine nucleotide and oxidation of either succinate or of cytochrome as indicated by Fig. 5*B*. A 'phosphate potential' of a few thousand calories produces considerable oxidation of cytochrome in substrate-free mitochondria (see below). For portions of the electron transfer system, such as those postulated for active transfer[4], anaerobiosis would not be required, only an appropriate 'phosphate potential'. It is apparent that the stoichiometry of the conversion from adenosine triphosphate to electrons is not yet sufficiently favourable to merit serious consideration of the mitochondrial process as an explanation for the very high efficiency observed in gastric secretion under certain conditions[6], although the principle of conversion of the energy of adenosine triphosphate to electron transfer is proved. However, in mitochondria we would not expect a ratio of adenosine triphosphate/electron exceeding that of the phosphorylation efficiency. Our stoichiometric data ($\sim$ 1 ATP/Fe) may give a low value because a portion of the adenosine triphosphate may be (1) used to affect other carriers that are oxidized and (2) split directly without its utilization in cytochrome reactions (see later).

The response of cytochromes of photosynthetic bacteria to illumination is characteristically an oxidation of cytochrome[17,18], and in some circumstances a reduction of pyridine nucleotide[19,20]. Up to this point, it has been considered that such responses are direct consequences of the effect of the photochemically produced oxidant and reductant on a series of electron-transfer carriers. The results given here make it necessary to distinguish by further experimentation between the present hypothesis and the possibility that high-energy intermediates, produced in light-induced energy-conservation reaction, could indirectly influence a chain of electron-transfer carriers in the manner illustrated by these experimental results (cf. Fig. 2). One way of distinguishing in favour of a direct effect is by observation of cytochrome oxidation at liquid-nitrogen temperatures[22].

The phenomenon of 'water splitting' by cytochrome a_3 oxidized by adenosine triphosphate would not be unexpected in further investigations; so far we have observed that a considerable disappearance of absorption at 445 mμ occurs on addition of adenosine triphosphate to the dithionite-treated material, and is followed by a reappearance of this absorption band. It is possible that the reduction of cytochrome oxidized by adenosine triphosphate could be attributed in part to a 'water-splitting' reaction, as well as to the exhaustion of added adenosine triphosphate.

Oxidations in the Presence of Dithionite

It is remarkable that cytochrome oxidation induced by adenosine triphosphate can be observed in mitochondria in spite of the reducing power of sodium dithionite. Two explanations may be considered: (1) that adenosine triphosphate can oxidize cytochromes against the negative potential of dithionite; or (2) that dithionite does not penetrate the mitochondrial membrane (assuming that none of the cytochromes is accessible on the outside membrane of the mitochondria). In the case of the second alternative, it might be expected that no responses to adenosine triphosphate would be observed at longer times following the addition of dithionite. We have been able to observe the response to adenosine triphosphate after incubation of the mitochondria with dithionite for one and a half minutes. The lack of dithionite sensitivity recalls experiments on the light-induced oxidation of cytochromes of *R. rubrum* in the presence of a considerable excess of dithionite[23]. At the present time, we are inclined to favour explanation 2 in both cases, namely, that dithionite is acting exclusively as an oxygen absorber and not as a specific reductant of cytochromes.

Nature of the Electron Acceptor

While it is clear from Figs. 1, 2 and 3 that cytochromes are oxidized, it is important to consider the electron acceptor for this oxidation reaction. (Obviously either electrons or hydrogen atoms could be transferred.) In Fig. 2*A* the rates of cytochrome oxidation and pyridine nucleotide reduction indicate that diphosphopyridine nucleotide may be accepting about half its electrons from cytochrome oxidation, and that the remainder arise (1) from succinate oxidation or (2) from entry of adenosine triphosphate at another point in the respiratory chain to oxidize flavoprotein and ubiquinone. It is possible that both reactions occur. It is of special interest to note that in the absence of added succinate, only 30 per cent of the pyridine nucleotide is reduced, and in this case the electrons are presumably all from oxidations induced by adenosine triphosphate of other carriers. The fact that only a part of the pyridine nucleotide can be reduced by electrons transferred from adenosine triphosphate-induced carrier oxidation or from treatment of the mitochondria with a diphosphopyridine nucleotide-linked substrate[3] underlines the specific role of succinate in the reduction of the majority of mitochondrial diphosphopyridine nucleotide and supports the ideas of chemical or compartmentation differences of mitochondrial pyridine nucleotide[3].

In the absence of succinate, pyridine nucleotide can still act as an electron acceptor, although some other substance may be involved. Comparing the total of oxidized cytochromes and flavoprotein with the extent of reduction of pyridine nucleotide, more oxidized than reduced substances are found on a mole basis (1·5 : 1). However, the assay procedures should be refined, particularly that for ubiquinone, since absorption of adenosine triphosphate interferes with its assay by the spectrophotometric method[24].

The reaction kinetics of cytochromes *c* and *a* are of interest in contrast with those of pyridine nucleotide; the former resembles those of an enzyme substrate intermediate, the latter that of a product of an overall reaction. As indicated above, the difference in these kinetics is probably due to the fact that succinate supplies electrons for the reduction of a portion of diphosphopyridine nucleotide.

Speed of the Reaction

The speed of cytochrome oxidation caused by addition of adenosine triphosphate is fairly high, as illustrated by Figs. 1 and 2, but it is nevertheless only about 10 per cent of the rate of oxygen utilization obtainable in the phosphorylation of adenosine diphosphate (state 3) in pigeon-heart mitochondria. It can then be understood why the accumulation of adenosine triphosphate does not slow down respiration in oxidative phosphorylation, or at least change the state of the carriers[1,25]. In addition, phosphate + adenosine diphosphate inhibit the reverse reaction[26]:

$$\mathrm{ADP} + \mathrm{P}_i + X \rightleftharpoons \mathrm{ATP} + X \qquad (1)$$

Similarly, oxygen itself, which produces a high degree of oxidation of the carriers, inhibits the reverse reaction.

Thermodynamic Aspects

While the records of Figs. 1 and 2 show clearly that the system is not in equilibrium because of breakdown of adenosine triphosphate, it is of great interest to learn something of the [ADP] [P$_i$]/[ATP] values that are involved in the cytochrome oxidation. In Fig. 5*B* a preliminary value of 10^2 for half-oxidation of cytochrome *c* is obtained. If succinate is added, the value is greatly decreased, as would be expected from the increased reducing power. While conversion of these values to calories may not be meaningful, cytochrome *c* is half-oxidized in the absence of succinate at an [ADP][P$_i$]/[ATP] value corresponding to 3 kcal.

That some oxidation of cytochrome and some reduction of diphosphopyridine nucleotide can be observed under these conditions requires an explanation. While it is not the purpose here to present a comprehensive theory, it is suggested that a possible explanation for the oxidations and reductions over such a wide range of redox potentials with such large [ADP] [P$_i$]/ATP values is due to a reversal of electron transfer accompanied by an accumulation of the inhibited high-energy forms of the respiratory carriers. The latter reaction apparently proceeds to such an extent that the interaction of the components of the chain as a whole is sufficiently slow to enable the rapid interaction with adenosine triphosphate to control the redox-level of various couples in the chain. Thus the present work emphasizes the formation of inhibitory compounds on addition of adenosine triphosphate, whereas previous work emphasized the reversal of inhibition on adding adenosine diphosphate and inorganic phosphate[15].

Relation to Adenosine Triphosphatase Activity

In considering a difference between the 'conservative' breakdown of adenosine triphosphate in a reaction yielding oxidation of cytochrome, and reduction of pyridine nucleotide and a 'dissipative' breakdown of adenosine triphosphate without such conservation of energy, it is useful to consider the sequence of equations:

$$\mathrm{ATP} + X \rightleftharpoons \mathrm{ADP} + X \sim \mathrm{P} \qquad (2)$$

$$X \sim \mathrm{P} + \mathrm{I} \rightleftharpoons X \sim \mathrm{I} + \mathrm{P}_i \qquad (3)$$

$$X \sim \mathrm{I} \overset{\mathrm{DNP}}{\rightleftharpoons} X + \mathrm{I} \qquad (4)$$

Reaction 4 would correspond to 'dissipative' reaction and reaction 5 to a 'conservative' reaction:

$$X \sim \mathrm{I} + rfp + \mathrm{DPN} \rightleftharpoons \mathrm{DPNH} + fp + X + \mathrm{I} + \mathrm{H}^+ \qquad (5)$$

Cross-over Points for Adenosine Triphosphate Interaction

Reaction 5 is based on the evidence of Fig. 6, namely, that flavin oxidation and pyridine nucleotide reduction occur in the system inhibited by quinoline-N-oxide; both the flavin and the pyridine nucleotide are associated with the side pathway involved in succinate-linked reduction of pyridine nucleotide[2,25,27] There appears to be a cross-over point for the oxidation-reduction reaction induced by adenosine triphosphate, which corresponds to a cross-over point between reduced diphosphopyridine nucleotide and flavin observed in response to adenosine diphosphate under appropriate conditions[28]. We have not yet discovered suitable conditions for demonstrating clearly other cross-over points which would indicate further similarities in the interaction sites for adenosine tri- and di-phosphate, one being expected between cytochromes c and a, and another between cytochromes c_1 and b (refs. 13, 28). It is apparent from these results that the behaviour of cytochrome b differs sharply from that of cytochrome c, the latter being rapidly oxidized and cytochrome b being slowly reduced. Thus a second cross-over point exists in this region of the chain. It remains to determine the role of cytochrome c_1 in this transition by work on 'trapped' steady states at liquid-nitrogen temperatures.

So far, no indication has been obtained of a cross-over point between cytochrome c and a, although this, of course, does not signify that the interaction does not occur here[26]. Thus there is no evidence available at the present time which indicates that the sites at which adenosine triphosphate interacts with the respiratory carriers to give 'reversed' electron transfer differ from the sites at which adenosine diphosphate is phosphorylated to adenosine triphosphate by means of 'forward' electron transfer.

This investigation was supported in part by the National Science Foundation.

Note added in proof. In work "On the Reversibility of Oxidative Phosphorylation", an oxidation of cytochrome c and reduction of flavoprotein induced by adenosine triphosphate have been observed in aerobic suspensions of rat skeletal muscle mitochondria[29]. More recently, it has been suggested that the aerobic results indicate an inhibition of electron transfer and not its reversal[30].

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SUBMOLECULAR STRUCTURE OF THE NUCLEIC ACIDS

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THE determination of the molecular structure of nucleic acids, which reveals the nature and mutual arrangement of the molecular species forming the giant polymers, is one of the most important achievements of modern biochemistry. The next logical step is the determination of the sub-molecular structure of these acids, which reveals the electronic structure of the constituents and the nature of the bonds which unite them. The establishment of the principal features of this sub-molecular structure should provide the proper basis for the understanding of the essential chemical, physico-chemical and biochemical properties of the nucleic acids.

Among the methods available for this investigation are the theoretical procedures of quantum chemistry, such as the valence bond or the molecular orbital

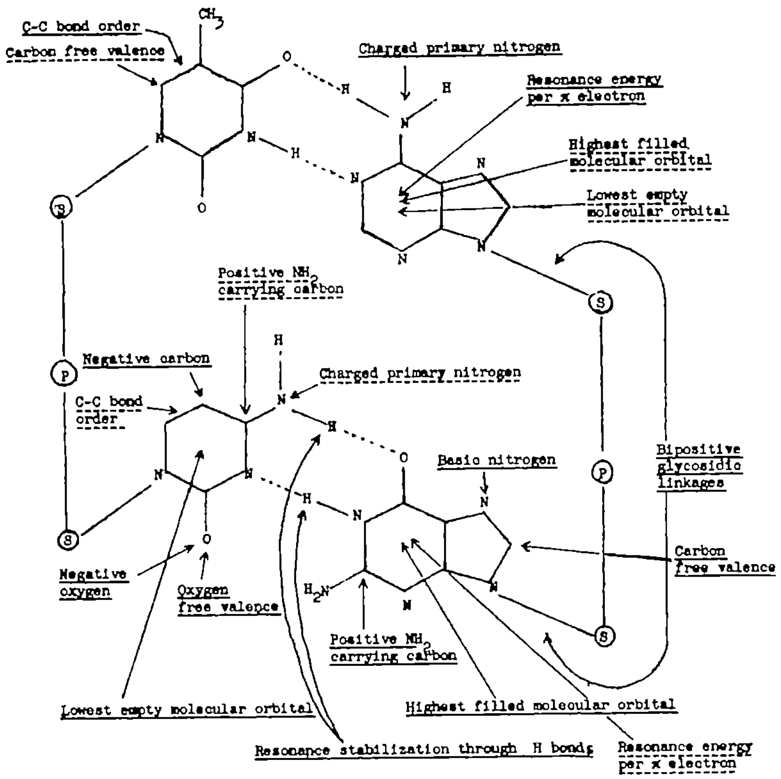
C-C bond order
Carbon free valence
CH_3
Charged primary nitrogen
Resonance energy per π electron
Highest filled molecular orbital
Lowest empty molecular orbital
Positive NH_2 carrying carbon
Negative carbon
C-C bond order
Charged primary nitrogen
Basic nitrogen
Bipositive glycosidic linkages
Carbon free valence
Negative oxygen
Oxygen free valence
Positive NH_2 carrying carbon
Lowest empty molecular orbital
Highest filled molecular orbital
Resonance stabilization through H bonds
Resonance energy per π electron
S
P

Fig. 1. Outstanding electronic indexes of the nitrogenous bases of nucleic acids

methods. This last method, in particular, has been shown in recent years to be of great value for the study of the electronic structure of biochemicals and of the mechanism, at the electronic level, of biochemical processes. It has been applied extensively, in our laboratory, to the detailed investigation of the electronic aspects of the structure of the nucleic acids and especially cf that of their purine and pyrimidine bases.

This work enables the determination of the values of the essential energetic and electronic indexes characteristic of the bases or of their constituent atoms or groups of atoms. It thus also renders possible the determination of the sites at which any particular index has its most significant value. Consequently, it enables the establishment of correlations between these outstanding values of the indexes and the essential chemical and physico-chemical properties of the nucleic acids.

The principal results obtained are summarized in Table 1.

A more pictorial representation of these general results is given in Figs. 1 and 2. They contain the fundamental base pairs as present in the nucleic acids (S = sugar, P = phosphate). Fig. 1 indicates the sites at which the essential theoretical indexes of electronic structure possess their most significant values. In fact, the indexes are underlined by a heavy line when placed at the site of their most significant value and by a broken line at their next most important site.

In Fig. 2, the same thing has been done for the different chemical or physico-chemical properties determined by these indexes. In some cases, indexes or properties have been indicated on Figs. 1 and 2 without being underlined. This signifies that these indexes or properties are important characteristics of the corresponding bases, but that it is not possible to ascertain and compare their relative values in the different units.

The advantage of tne schematic representation given in Figs. 1 and 2 is that it enables a very quick localization of the principal reactive centres of nucleic acids towards given types of reagents.

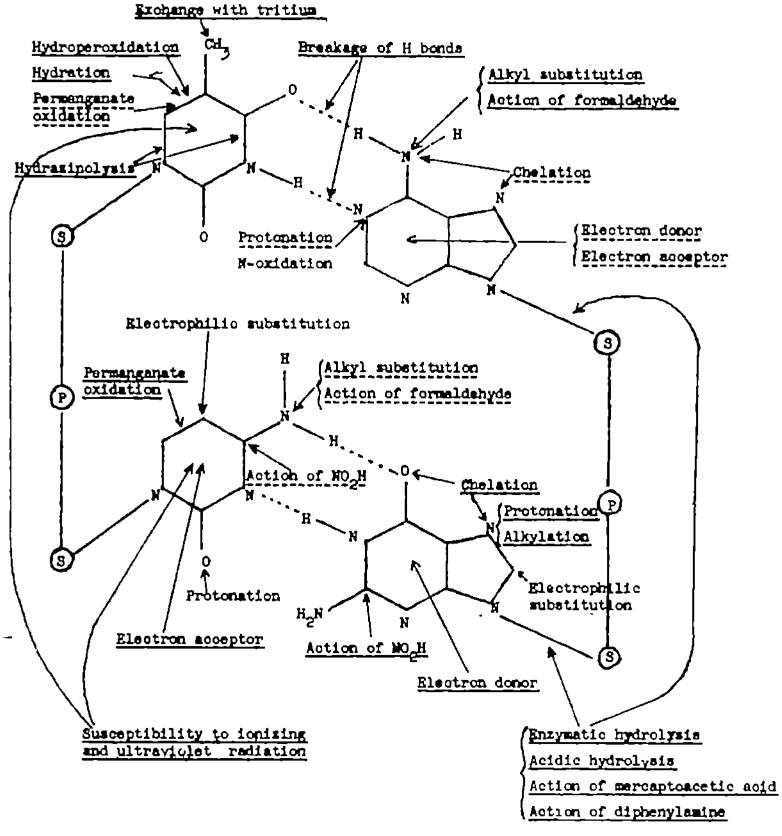
Exchange with tritium
Hydroperoxidation
Hydration
Permanganate oxidation
Hydrazinolysis
CH_3
Breakage of H bonds
Alkyl substitution
Action of formaldehyde
Chelation
Protonation
N-oxidation
Electron donor
Electron acceptor
Electrophilic substitution
Permanganate oxidation
Alkyl substitution
Action of formaldehyde
Action of NO_2H
Chelation
Protonation
Alkylation
Electrophilic substitution
Protonation
Electron acceptor
Action of NO_2H
Electron donor
Susceptibility to ionizing and ultraviolet radiation
Enzymatic hydrolysis
Acidic hydrolysis
Action of mercaptoacetic acid
Action of diphenylamine
S
P

Fig. 2. Principal chemical and physico-chemical properties of the nitrogenous bases of the nucleic acids

Table 1, FUNDAMENTAL ELECTRONIC INDEXES AND OUTSTANDING CHEMICAL AND PHYSICO-CHEMICAL PROPERTIES OF THE NUCLEIC ACIDS

The electronic index	Its manifestation
Resonance energy. The greatest resonance energy per π electron is that of the adenine ring. The next one is that of guanine Resonance stabilization through hydrogen bonds is greater for the guanine-cytosine pair than for the adenine-thymine pair[1,2].	Resistance of purines and in particular of adenine to ionizing and ultra-violet radiations[3]. Higher temperature of denaturation of nucleic acids rich in guanine-cytosine pair, than of those rich in adenine-thymine pair[3].
Highest filled molecular orbital. The highest filled molecular orbital is the highest in guanine followed by adenine[4,5].	The electron donor properties of purines, visible in their tendency to form charge-transfer complexes with different electron acceptors[4,5].
Lowest empty molecular orbital. The lowest empty molecular orbital is the lowest in cytosine, followed by adenine[4,6].	The electron acceptor properties of these two bases, visible in their tendency to trap electrons[6].
Basicity of ring nitrogens. In the nucleic acids the most basic nitrogen is predicted to be N_7 of guanine. In adenine the essential basic centre should be N_1 (in nucleic acid adenine it may be N_7)[1,2].	Protonation and alkylation by alkylating agents occur at N_7 of guanine and N_1 of adenine[7-9]. N-oxidation occurs at N_1 of adenine[10]. Alkylation of nucleic acids by alkylating agents occurs essentially at N_7 of guanine[11-13]. The principal site of chelation by metals is between N_7 and the O of guanine[14].
Charge of the primary amino-nitrogen. Because of the delocalization of their lone pairs the nitrogens of the NH_2 groups are formally positive. The most charged among them (the less positive) is that of adenine, followed by that of cytosine[1].	Direct alkyl substitution at the amino-group and the reaction with formaldehyde occur essentially on the NH_2 groups of adenine, followed by that of cytosine[15-17].
Charge of the carbon atoms carrying the amino-groups. These carbon atoms are formally positive. The most positive among them is that of guanine, followed by that of cytosine.	Deamination of deoxyribonucleic acid by NO_2H occurs essentially on guanine, followed by cytosine[18].
Charge of the oxygen atoms. The most negative oxygen is that of cytosine[1].	The oxygen of cytosine may be the primary site of protonation of undenatured deoxyribonucleic acid[19].
Carbon-carbon bond order. The greatest C-C bond order is that of the 5-6 bond of thymine, followed by the 5-6 bond of cytosine[2].	Hydroperoxidation under the action of ionizing radiations and hydration upon photolysis occur essentially at the C_5–C_6 bond of thymine[2]. Permanganate oxidation occurs essentially at the C_5–C_6 bond of cytosine, followed by that of thymine[20].
Indexes of unsubstituted C atoms. C_8 of guanine has the greatest free valence, C_5 of cytosine the greatest formal negative charge[1,2].	Both carbons are sites of electrophilic substitutions[21].
Bipositivity of the C-N glycosidic linkage. The formal positive charge of the sugar carrying nitrogen and the bipositivity of the glycosidic linkage are greater for the purines than for the pyrimidines[22].	Preferential reactivity of the glycosidic linkages of the purines in enzymic or acidic hydrolysis[22] and towards mercaptoacetic acid and diphenylamine[23,24].

In this respect, one of the striking features of Fig. 2 is that none of the quoted reactions appears heavily underlined for the adenine ring. (It is only the transformations which are concerned with its extracyclic NH_2 group which appear as occurring with particular ease at that molecule.) The relatively great stability of adenine towards external agents of any type is thus immediately visible after a simple glance at Fig. 2. This impression is still substantiated by a glance at Fig. 1 on which the only characteristic of the adenine ring which deserves the heavy underlining is its relatively great resonance energy. The particular part played by adenine in biochemistry has been tentatively linked with its particularly pronounced thermodynamic and kinetic stability[3].

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DETECTION OF LIVER PROTEINS IN CIRCULATING BLOOD

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THE release of proteins from rat liver during perfusion of this organ with rat blood[1] can be demonstrated by means of rabbit antiserum against a homogenate of whole rat liver using the gel precipitation method of Ouchterlony[2]. To avoid precipitation lines due to the rat plasma proteins, the antiserum was always adsorbed with rat plasma before use. When the antiserum was allowed to react with the liver homogenate against which it was formed, 8–10 lines were obtained. Under similar conditions, plasmas from blood which had been used for perfusion of a rat liver for 4–5 hr. gave 2–6 lines. As may be seen in Fig. 1, both the number and the intensity of the lines produced by plasmas from different liver perfusions varied very greatly. The experiment (Fig. 1) also shows immunological identity, or partial identity, between proteins of the liver homogenate and those liberated during perfusion of the rat liver.

Altogether, plasmas from thirteen perfusion experiments were examined by means of the antiserum. In eight of these, heparin was present as the anticoagulant. In three the freshly drawn blood was mixed with 0·167 vol. of 3·8 per cent sodium citrate. After centrifugation the plasma was clotted by the addition of calcium ions. The serum thus obtained was then re-mixed with the red cells. In the last two experiments no sodium citrate was used, the blood being centrifuged in plastic tubes, separated and the plasma allowed to clot. After removal of the fibrin the serum was re-mixed with the red cells. In the last five experiments the only fibrinogen or fibrin which can have been present will have been that formed by the liver during the course of the perfusion.

When heparin was used the precipitation lines formed by the antiserum as judged by eye, although very variable in intensity, were on average much heavier than those which were formed after addition of citrate and removal of fibrin. The two plasmas which appeared to contain the smallest amounts of solubilized liver protein were from experiments of the latter kind. On the other hand, in another experiment of this kind, heavier precipitation lines were observed. Both the plasmas which had been defibrinated and then used without added sodium citrate gave rather heavy precipitation lines with the antiserum. In one of these experiments the perfusion-rate fell progressively and, at the end, material which seemed to be fibrin was found adhering to the glassware. Although experimental conditions for the perfusion of rat livers which will completely prevent the solubilization of even small amounts of liver material have not yet been completely defined, the above results with defibrinated blood containing citrate suggest the usefulness of such media.

Because of the apparent readiness with which certain components appeared in the plasma during perfusion of the isolated liver, conditions which would lead to release of proteins from the liver *in vivo* were sought. Plasmas were examined from rats subjected to: (1) Repeated injection with large amounts of indian ink over four days (for details, see Freeman, Gordon and Humphrey[3]). (2) Anaphylaxis; a rat previously sensitized by means of hæmocyanin in Freund's adjuvant was injected intravenously with 2 mgm. of alum precipitated hæmocyanin. Two hours after the commencement of severe, but non-fatal anaphylaxis, the animal was bled. (3) Intravenous injection of the lipopolysaccharide endotoxin of *E. coli* 08 (from Prof. O. Westphal). Whereas no effect could be detected as a result of either of the first two treatments, plasma obtained 6 hr. after the injection of 1 mgm. of the endotoxin already yielded a single precipitation line. After a further 16 hr. the plasma from the same animal gave three lines. The rat which showed minimal signs of prostration was then killed. The liver showed many pale spots.

The identity or otherwise of the liver components released into the plasma *in vivo* by the endotoxin with those appearing after perfusion of a liver for several hours was also examined. In all experiments continuity of the precipitation lines formed between the antiserum and plasmas from each kind of experiment was observed. Apparently no components present in one plasma were completely absent from the other. On the other hand, large quantitative differences between certain components were noticeable.

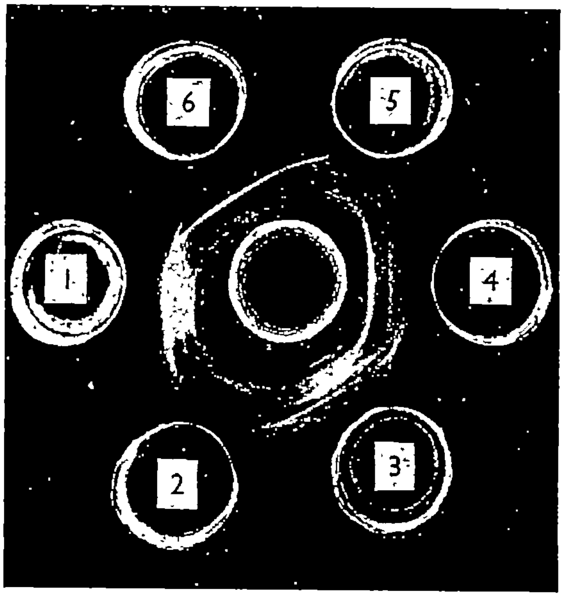

Fig. 1. Centre hole: antiserum produced in rabbits by injection of rat liver homogenate treated with Freund's adjuvant. The antiserum has been absorbed by subsequent addition of rat plasma. (1) and (3) Rat liver homogenate diluted with 9 vol. of saline; (5) rat liver homogenate diluted with 19 vol. of saline; (2) plasma from rat blood used for perfusion of a rat liver for 4 hr.; (4) plasma from rat blood used for perfusion of a rat liver for 5 hr.; (6) plasma from rat blood used for perfusion of a rat liver. For this perfusion the plasma protein concentration was raised to 104 mgm. per ml. Heparin was used in each of these perfusion experiments

In order to obtain information on the amount of material passing from the liver into the circulating blood during a perfusion experiment, comparison was made between the precipitation lines given with the antiserum by appropriate dilutions of a saline extract of rat liver and by samples of plasma from blood after use for perfusion. Appropriate dilutions of the liver extract and dilutions of the perfusion plasma were arranged alternately around a central well. By observing which samples gave precipitation lines of similar intensity and position, it was possible to estimate the dilution of liver extract containing amounts of particular liver components corresponding to those in the perfusion plasma.

Three samples of plasma taken at the end of three liver perfusion experiments were thus investigated. The first was from a perfusion in which heparin was present and in which the plasma protein concentration had been increased above normal. This plasma was found to contain at least 3·5 mgm. per ml. of liver protein. The two other plasmas, from experiments in which defibrinated blood containing sodium citrate had been used, gave 0·23 and 2·1 mgm. per ml., respectively. Considered in relation to the total weight of protein in the perfused liver, these figures indicate that at least 0·5–5 per cent of originally intracellular material had appeared in the circulating plasma.

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AFFINITY OF ORGANIC COMPOUNDS FOR FREE ELECTRONS WITH THERMAL ENERGY: ITS POSSIBLE SIGNIFICANCE IN BIOLOGY

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MANY molecular species react with free electrons to form stable negative ions. In the classical physical researches on the behaviour of free electrons in gases[1-3], the study of negative-ion formation was restricted to a few simple substances such as water, oxygen and the halogens. More recently, a technique has been developed for observing the electron affinity of volatile organic substances generally[4]; this technique was primarily intended as a qualitative analytical method for the identification of minute quantities of volatile organic compounds. It was based on the observation that the electron affinity of a homologous series of compounds was determined principally by the affinity of the common functional group and was relatively independent of the hydrocarbon moiety. The establishment of this method requires measurement of the electron affinities of a wide range of compounds at various electron energies; this is a lengthy task and not yet completed. In the course of this investigation it has been observed that the affinities of compounds for electrons with thermal energies are frequently associated with their biological activity, particularly in those systems thought to involve electron transport. For example, among the few simple aliphatic compounds with an extreme affinity for electrons are the intermediates on the oxidation pathways of the living cell, such as pyruvate and fumarate. The potential interest of this and other similar comparisons between biological activity and electron affinity encouraged the preliminary report which follows. It describes briefly the methods used to measure the affinities of organic substances for thermal electrons and includes a discussion of the general problem of electron transport in the living cell in the light of these new measurements.

Physical basis and experimental method. Free electrons and molecules react to form stable negative ions by either of the two following processes:

$$e^- + AB = (AB)^- + \text{energy} \qquad (1)$$

$$e^- + AB = A + B^- \pm \text{energy} \qquad (2)$$

In the first reaction, the energy released may escape as radiation or be shared with other molecules during subsequent collisions. The energy balance of the second reaction is determined principally by the following factors: the relative kinetic energies of the reactants before and after collision, the energy released by the formation of a negative ion from a constituent atom or radical of the compound and the energy released or required to dissociate the molecule. Whichever of these reactions takes place, electrons are lost and stable negative ions formed. The probability of electron capture by a given compound depends on the elementary composition and structure of its molecules and on the kinetic energy of the colliding electrons. In the analytical application, it is convenient to observe the spectrum of affinity of a given molecular species at different electron energies. In the present work the affinities of different substances were observed using electrons in thermal equilibrium with gas mixtures at temperatures of 20°–200° C. This range of energies is presumably similar to that of free electrons, should they so exist, in biological systems.

The apparatus used is illustrated as a schematic diagram (Fig. 1). It consisted of a plane parallel ionization chamber with an electrode separation of 1 cm., and was identical in construction to that described for another purpose[5]. The chamber was swept continuously by a stream of clean dry argon containing 0·3 per cent of hydrogen at a rate of 100–1,000 ml. per min. at atmospheric pressure. Electrons were set free in the chamber by means of soft β-radiation from a sheet of metal coated with a thin layer of titanium tritide; this radiation source also served as the cathode of the chamber. The density of free electrons in the chamber was measured by applying to the anode, every 10 μsec., a rectangular pulse 50 V. in amplitude and 0·5 μsec. in duration. The duration of this sampling pulse was chosen to be just sufficient to collect all the electrons set free in the chamber; in other words, capable of withdrawing a saturation current from the chamber. Such a pulse duration was, however, much too short to collect any of the relatively immobile negative molecular ions. The contribution to the total current-flow of electrons liberated during the application of the pulse was only 5 per cent; the loss of electrons, when vapours capable of capturing them were present in the chamber, was therefore observed effectively under field-free conditions and when the energy of the electrons present was thermal. The vapours of the test substances were introduced into the gas stream flowing to the chamber after their passage through an efficient 100-ft. capillary tube chromatograph column. This precaution was found essential since few of the substances tested were free of electron-capturing contaminants. The concentration of vapour in the gas stream was measured by means of a photo-ionization detector[6] connected in parallel with the chamber for observing electron capture. The current-flow from the two ionization chambers was amplified by electrometer amplifiers and recorded using two separate potentiometric recorders.

Fig. 2 illustrates a typical measurement in which the emergence of several compounds in sequence from the chromatograph column is displayed as chromato-

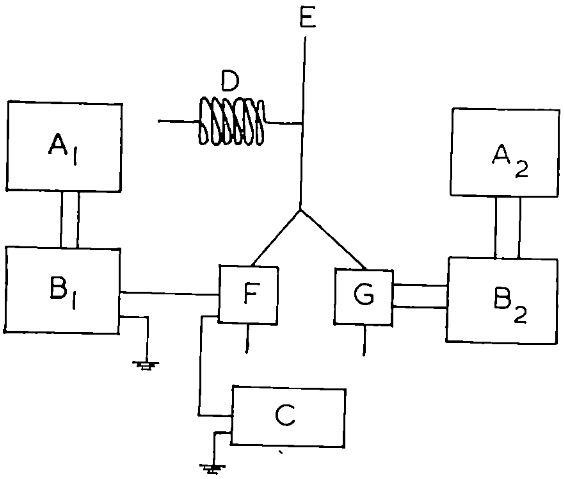

Fig. 1. Schematic diagram of the apparatus used for the measurement of relative electron affinities. A_1 and A_2 potentiometric recorders, B_1 and B_2 electrometer amplifiers, C a pulse generator, D a capillary tube chromatograph column, E inlet for diluent gas, F the ionization chamber for observing electron capture and G a photo-ionization detector for the measurement of concentration of test vapour

grams. The lower chromatogram shows the vapour concentrations of the test substances and the upper shows the extent of electron capture. The small vapour concentrations of the test substances, ethyl pyruvate and dimethyl fumarate, are seen to capture electrons much more readily than the large vapour concentration of the reference substance, chlorobenzene. Also, the test substance, diethyl succinate, is seen to have little or no affinity for electrons. The relative electron affinities of the different substances tested were compared in terms of the ratio of the change of current in the two ionization chambers and are expressed always as relative to the reference standard, chlorobenzene. Whenever possible, the quantity of test substance used was adjusted so that the change in current in the electron-capture chamber did not exceed 10 per cent of the total current. Above this value the change of current is no longer linearly related to vapour concentration. To meet this need, quantities of between 10^{-6} and 10^{-9} gm. were applied to the column. It must be emphasized that the electron affinities reported here may be as much as 30 per cent in error. Uncertainties arise principally in the measurement of vapour concentration. It is not yet possible to apply precisely known amounts of test substances to the efficient capillary tube column. Also, the response of the photo-ionization detector used to measure vapour concentration is not yet known with precision. This detector was chosen for this application because it is the only available sensitive ionization detector the response of which is not adversely affected by substances with a high affinity for free electrons.

Relative electron affinities of the substances tested. Table 1 lists the electron affinities, relative to chlorobenzene as unity, of the strongly electron-attracting substances tested; also shown are the principal electron-attracting substituents or structures of these substances and the electron affinities of comparable weakly attracting compounds.

The great majority of simple aliphatic organic compounds possess a slight or negligible affinity for free electrons of thermal energy. Those which do capture electrons strongly have one or more of the following structures or substituents in their molecules, which henceforth will be called 'electrophores': (1) —CO.CO—, (2) —CO.CH : CH.CO—, (3) the quinone structure, (4) $—NO_2$, (5) $—X$, where X is one or more halogen atoms. With substitution by halogens affinities fall in the order: $I > Br > Cl > F$; multiple substitution by halogen- or nitro-groups greatly enhances the affinity for electrons.

It is probable that other more or less complex structures can function as electrophores. Thus, although simple conjugated and aromatic hydrocarbons such as butadiene, benzene, naphthalene and phenanthrene show little affinity, more complex conjugated hydrocarbons, including *cyclo*-octatetrene, azulene, diphenyl butadiene and anthracene, all show a marked affinity for free electrons. It appears also that [—CO.] in the electrophores listed above can be replaced by a phenyl radical without great loss of affinity; a substitution of nitrogen in place of carbon can also be made. It must be emphasized, however, that affinity of a high order, greater than ten, is rare among the simpler organic compounds. An affinity of this order has not been found during the examination of 430 members of the following classes of organic compounds: saturated, ethenoid and ethinoid hydrocarbons, ethers, esters, alcohols, carboxylic acids, aldehydes, ketones, nitriles, amines and, with the exceptions listed in Table 1, compounds with two or more of these groups. Although weakly attractive to thermal electrons, the compounds just listed do possess a sufficient affinity for analytical purposes.

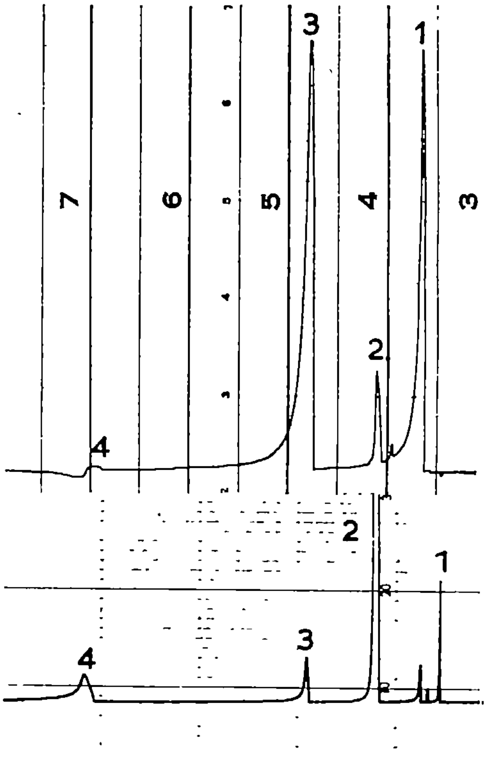

Fig. 2. Measurement of electron affinity using the apparatus indicated in Fig. 1. The lower chromatogram shows the concentrations of vapours emerging from the chromatograph column, the upper chromatogram the capture of electrons by the same vapours measured simultaneously. The test substances are ethyl pyruvate (1), chlorobenzene (2), dimethyl fumarate (3) and diethyl succinate (4)

Table 1. AFFINITIES FOR THERMAL ELECTRONS OF COMPOUNDS OF BIOLOGICAL INTEREST

All values are relative to the electron affinity of chlorobenzene, which is taken to be unity

Compounds with high electron affinity	Electrophore	Affinity	Compounds with low electron affinity	Affinity
Ethyl pyruvate	—CO.CO—	1,700	Ethyl acetate	< 0 01
Diacetyl	—CO.CO—	2,000	Acetylacetone	1·2
Dimethyl oxalacetate	—CO.CO—	1,300	Ethyl acetoacetate	0·7
Diethyl fumarate	—CO CH : CH CO—	1,500	Ethyl acrylate	0·1
Diethyl maleate	—CO CH : CH CO—	1,700	Diethyl malate	0·01
			Diethyl succinate	< 0·01
Quinone	*Q*	5,000		
Dimethylnaphthaquinone	*Q*	4,100	Hydroquinone	0·1
Benzaldehyde	—CO *P*	48	Benzyl alcohol	0·05
Cinnamaldehyde	—CO CH : CH *P*	310		
cis-Stilbene	*P* CH : CH *P*	1	Styrene	< 0·01
trans-Stilbene	*P* CH · CH.*P*	4		
Acetamide	—CO NH.	12	Methyl amine	0·01
Azobenzene	*P*.N : N.*P*	9	Aniline	0 1
Diacetyldihydropyridine	*Q*	4,000	Pyridine	0 02
Anthracene		12	Phenanthrene	0 05
Azulene		340	Naphthalene	< 0 01
Cyclo-octatetrene		210	Cyclopentadiene	< 0·01
Carbon tetrachloride	—Cl	7,000	Ethane	< 0·01
Chloroform	—Cl	800		
Hexachlorobenzene	—Cl	1,100	Benzene	< 0 01
Chlorobenzene	—Cl	1		
Bromobenzene	—Br	6		
Iodobenzene	—I	870		
Nitrobenzene	—NO_2	390		
Dinitrophenol	—NO_2	1,450		

P, phenyl radical. *Q*, quinone structure.

At present no information is available concerning the end products of the reaction between the compounds tested and free electrons. It is probable, however, that capture is followed by dissociation with the halogen- and nitro-compounds. The hydrocarbons and perhaps some CHO compounds are, on the other hand, likely to form stable negative molecular ions.

Relevance to biological systems. It has been suggested that protein may show semi-conducting properties important in its normal function[7]. More recently, evidence has become available to suggest that in photosynthesis[8,9] and in the transfer of energy in mitochondria[10,11], free electrons play an important part. In the light of these suggestions it is of interest that the electrophores, —CO.CO—, —CO.CH : CH.CO— and the quinone structure are all closely associated with those systems thought to be important in electron transfer. Thus, in the process of oxidative phosphorylation proceeding from pyruvate through the Krebs cycle to carbon dioxide each alternate intermediate has one or other of the two electrophores, —CO.CO— and —CO.CH : CH.CO—. The first is found in pyruvate, oxalacetate, and ketoglutarate; the second in *cis*-aconitate and in fumarate. The remaining intermediates are found in the form of their esters, or would be thought to have but little affinity for free electrons. The 'inert' intermediates, including succinate, malate, acetate, citrate and *iso*citrate, are always interposed between the electron-attracting intermediates. Two other substances thought to be important in the transport of electrons in the living cell are coenzyme *Q* and diphosphopyridine nucleotide (DPN). The former has the quinone electrophore; it is conceivable that the structure of DPN includes an electrophore as yet unidentified.

The presence in the electron transport system of the living cell of an ordered distribution of so many of the few simple organic substances likely to have a high affinity for free electrons seems to be more than coincidental. It is also remarkable that among the few remaining substances with a similar high affinity for free electrons are several which are able to disturb the process of oxidative phosphorylation, such as dinitrophenol and certain halogen-containing compounds. The significance of the association between electron affinity and biological function depends on the assumption that the electrophores are as active in the relevant compounds in their natural state as they are in the volatile esters tested. If this assumption is justified, then the observations suggest the following unifying hypothesis: electron transport in the living cell proceeds through an ordered sequence of reversible electron traps. The introduction of foreign, especially irreversible, trapping agents is harmful because of their ability to remove electrons from the normal sequence. The term reversible trap refers to a compound which after capturing an electron changes either physically or chemically to a second compound the affinity of which is slight, and so releases the electron for the next trap in the sequence. To illustrate this point, fumarate could be considered to capture an electron and the negative fumarate ion then reacts with water to form a malate negative ion; the weak affinity of malate for electrons would encourage their transfer to the next trap in the sequence, possibly DPN.

The hypothesis that electron trapping in the living cell is a purposeful process offers explanations for a number of apparently intractable problems in biological electron transport. A few of these problems, particularly those concerned with the mechanism of action of toxic substances able to disturb the normal process of electron transport, are briefly discussed as follows:

In descriptions of the mechanism of photosynthesis, it has repeatedly been stressed that some process is required to explain the rapid removal of primary electrons before they can recombine with the positive charges simultaneously produced by photon impact[12]. The trapping mechanism just described would meet this need.

The volatility of the thyroid hormones is too low to measure their electron affinity by the methods just described. It is tolerably certain, however, that these compounds, which contain three or more iodine atoms, are potent electron traps. The possibility that the thyroid hormones owe their ability to moderate the process of oxidative metabolism to their high affinity for free electrons is supported by the following circumstantial evidence: the biological activities of trichloro-, tribromo- and tri-iodo-thyronine[13] are respectively: 1, 37 and 420. The electron affinities of chloro, bromo- and iodo-benzene are respectively: 1, 6, 370. Although the structures of the thyronines are very different from those of the halogen-substituted benzenes, the comparison is justified by the fact that the electron affinity conferred by halogen substitution is frequently independent of the nature of the molecule substituted; provided, of course, no other electrophores are also present. The resemblance between the biological activity of the thyroid hormones and that of dinitrophenol is further evidence in support of this interpretation.

Another reaction in biochemistry thought for some time to involve electron transfer is that between carcinogenic hydrocarbons and protein. A stumbling block to the understanding of the nature of this reaction has been the persistently held assumption that the hydrocarbon is the donor and the protein the acceptor of electrons; this has been difficult to reconcile with theory or observation. It was suggested tentatively[14,15] that these difficulties could be resolved if the hydrocarbon were in fact the acceptor of electrons. The unpopularity of the notion of a hydrocarbon so behaving in that way seems to have prevented the general acceptance of the suggestion. However, as shown in Table 1, complex hydrocarbons are very ready to function as electron acceptors. Because of their slight volatility, it has not yet been possible to test the carcinogenic hydrocarbons themselves, although it is of interest that stilbene and azobenzene both are able to capture electrons and give rise to simple derivatives with carcinogenic activity.

In general, where a similar toxic action is shared by compounds differing only in substitution with halogen- or nitro-groups, and where a disturbance of electron transport is thought to occur, then the toxicity of the compounds could be due to their ability to trap electrons. One important difference between normal intermediates which can trap electrons and the toxic substances could be in the type of reaction which takes place. Trapping of electrons is potentially most harmful when it is irreversible; in these circumstances, the potential energy of the captured electron may be completely removed from the system. Furthermore, if dissociative capture occurs, and it is likely to occur with halogen- and nitro-compounds, then a highly reactive and potentially harmful free-radical may be formed. In addition to those classes of toxic agent already mentioned, other substances which may function in a similar way include halogenated insecticides, inhibitors of plant growth, certain fungistatic iodo- and nitro-compounds[16] and certain antibiotics such as chloramphenicol. Some of the limitations of the evidence provided from measurements of electron affinity arise from the need to conduct the observations on volatile derivatives in the gas phase. There is no reason in principle why similar measurements to those described should not be made in the liquid or even solid phases. The solution of the practical problems in this direction will greatly extend the range of measurement of this potentially important molecular property, and perhaps provide the evidence necessary for the confirmation or denial of the hypotheses outlined in this article.

I am indebted to my colleagues T. Nash and N. L. Gregory for the stimulus of their suggestions.

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AGGREGATION, VARIANCE AND THE MEAN

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IN populations where the individuals are distributed at random, that is, are independent of each other, the variance (s^2) at each population density is equal to the mean (m). Individuals in natural populations are not, however, independent of each other; mutual attraction leads to aggregation which makes variance more than the mean ($s^2 > m$), and mutual repulsion leads to regularity which makes variance less than the mean. Hence, in Nature, true randomness is only one of a continuous series of possible distributions (both in a spatial and a statistical sense) from regular to highly aggregated, and is consequently rare.

This property of natural populations, to show some degree of aggregation, is commonly thought to be highly specific; for example, soil zoologists know that leather-jackets are 'more highly aggregated' than wireworms, yet the meaning of this is intangible, because no consistent measure of aggregation is at present available. In addition, many kinds of statistical analysis require that the variance should be made independent of the mean and there is no rational transformation available covering all grades of aggregation. Aggregation has been measured most widely using k in the expression:

$$s^2 = m + km^2 \qquad (1)$$

derived from the negative binomial distribution. Unfortunately k is not always independent of m (ref. 5), and it is plainly desirable that an index of population structure should be the same at different population densities, unless some actual change in behaviour is involved.

In all the sets of samples I have examined, variance appears to be related to the mean by a simple power law; variance is proportional to a fractional power of the mean:

$$s^2 = am^b \qquad (2)$$

where a and b are characteristic of the population in question. This is most clearly demonstrated by a log × log plot of s^2 on m as in Fig. 1, which includes some classic population surveys. Table 1 gives a and b for these and other populations, namely, virus lesions, macro-zooplankton, worms and symphylids in soil, insects in soil, on plants and in the air, mites on leaves, ticks on sheep and fish in the sea.

Table 1

	Name	Site and sample	Range of m	Range of s^2	N	a	b	Transformation	Observer (refs. in brackets)
1	Shellfish on seashore, *Tellina tenuis* da Costa, Eulamellibranchiata : Mollusca	Sand, 63 units, various sizes	0·72–45·7	0·49–8·0	5	0·50	0·70	$x^{0·65}$	Holme (13)
2	European chafer larvæ, *Amphimallon majalis* Raz. (= *Melolontha melolontha* L.), Coleoptera : Insecta	Pasture soil, 25 units, each 1 ft. sq.	0·20–9·72	0·26–14·93	75	1·15	1·07	$x^{0·46}$	Burrage and Gyrisco (6)
3	Flying insects, various orders : Insecta	Open air, 16–104 units aerial density	1·9–238	1·7–606	24	1·0	1·17	$x^{0·41}$	L. R. Taylor (unpublished)
4	Wireworms, *Agriotes* spp. mainly *obscurus*, Coleoptera : Insecta	Grassland soil 20 units, 4 in. cores	0·20–4·65	0·40–17·80	2,272	2·75	1·19	$x^{0·40}$	Yates and Finney (23)
5	Wireworms, *Agriotes* as above	Arable land soil 20 units, 4 in. cores	0·20–4·65	0·39–22·50	525	2·85	1·26	$x^{0·37}$	Yates and Finney (23)
6	Wireworms, *Limonius* spp., Coleoptera : Insecta	Arable land soil, 175 units, each 1 ft. sq.	0·39–10·89	0·58–60·42	24	2·0	1·33	$x^{0·33}$	Jones (5, 14)
7	Gall midge larvæ, *Jaapiella medicaginis* (Rub.), Diptera : Insecta	Lucerne field soil, 10 units, 4 in. cores	0·22–5·6	0·19–13·82	12	1·3	1·33	$x^{0·33}$	Heath (12)
8	Spruce budworm larvæ, *Choristoneura fumifera* (Clem.), Lepidoptera : Insecta	Fir foliage, 25 units, larvæ/twig	0·48–15·04	0·343–51·86	50	1	1·40	$x^{0·30}$	Waters (4, 22)
9	Virus lesions, tobacco necrosis virus	Bean leaves, 4 units, lesions/half leaf	15·38–237·56	65·13–3,265·80	120	1	1·40	$x^{0·30}$	Kleczkowski (5, 15)
10	Colorado beetle adults, *Leptinotarsa decemlineata* Say., Coleoptera : Insecta	Potato foliage, 2,304 counts : insects/2 ft. row	1·0–12·1	1·4–37·7	16 16 5	1	1·48	$x^{0·26}$	Beall (2, 3, 5, 19)
11	Japanese beetle larvæ, *Popillia japonica* New., Coleoptera : Insecta	Soil, 10,000 units each 1 ft. sq.*	2·76–1,914	5·90–210,000	36	1·3	1·52	$x^{0·24}$	Fleming and Baker (10)
12	Macro-zooplankton	Water, net collection, slide count, 10 areas	95–1,750	1,444–194,480	4	1·0	1·57	$x^{0·21}$	Littleford, Newcombe and Shepherd (16)
13	Macro-zooplankton	As above, 50 areas	93–1,822	169–18,769	4	0·14	1·57	$x^{0·21}$	Littleford, Newcombe and Shepherd (16)
14	Ticks, *Ixodes ricinus* L., Acarina : Arachnida	Sheep, 20–86 units, ticks/sheep	0·84–85·6	2·5–2,292	10	1·0	1·66	$x^{0·17}$	Milne (18)
15	Enchytraeid worms, mainly *Fridericia bisetosa* (Lev.), Enchytraeidae : Annelida	Pasture, 60–150 units, 3·6 cm. cores	40·8–381·5	441–29,584	4	1·0	1·66	$x^{0·17}$	Nielson (20)
16	Corn borer larvæ, *Pyrausta nubilalis* (Hubn.), Lepidoptera : Insecta	Maize stalks, 2 stage†	6·7–970	88·5–180,220	1,054	3·0	1·66	$x^{0·17}$	Meyers and Patch (17)
17	Thrips, *Thrips imaginis* (Bagnall), Thysanoptera Insecta	Rose flowers, 20 units, thrips/rose	20·6–137·5	223–7,900	16	1·0	1·80	$x^{0·10}$	Davidson and Andrewartha (8)
18	Leather-jackets, *Tipula* spp., Diptera : Insecta	Soil, 2 units, Nos./sq. ft.	3·10–63·50	3·5–409·0	36	0·2	1·85	$x^{0·07}$	Bartlett (1, 5)
19	Earthworms, all stages, *Allolobophora chlorotica* (Sav.), Oligochaeta : Annelida	Grassland, 4 units, 18 in. sq.	2·8–71·3	2·9–1,410	54	0·2	2·00	$\log x$	Gerard (11)
20	Red spider mite, eggs and adults, *Metatetranychus ulmi* (Koch), Acarina : Arachnida	Apple leaves, 20 units, mites/leaf	8·5–216	90–55,100	162	0·4	2·19	$1/x^{0·10}$	Daum and Dewey (7)
21	Haddock, *Melanogrammus aeglefinus*, Gadidae : Pisces	Sea, 4–47 units, Nos./trawl	4·09–288	39·1–1,230,500	15	1·0	2·35	$1/x^{0·18}$	C. C. Taylor (5, 21)
22	Earthworms, all stages, *Allolobophora caliginosa* (Sav.), Oligochaeta : Annelida	Grassland, 4 units, 18 in. sq.	4·3–44·8	4·0–500	42	0·05	2·54	$1/x^{0·27}$	Gerard (11)
23	Symphyla, *Symphylella* spp., Symphyla : Myriapoda	Various soils, 60–120 units, 2½ in. cores	4·5–31·8	0·49–690	5	0·06	2·75	$1/x^{0·38}$	Edwards (9)
24	Symphyla, *Scutigerella* spp., Symphyla : Myriapoda	Various soils, 60–140 units, 2½ in. cores	1·3–31·4	0·64–1,250	6	0·035	3·08	$1/x^{0·54}$	Edwards (9)

* Units combined to cover areas varying in size from 1 sq. ft. to 100 sq. ft. † Larvæ/stalk × infested stalks/100 : this introduces some bias in the variances.

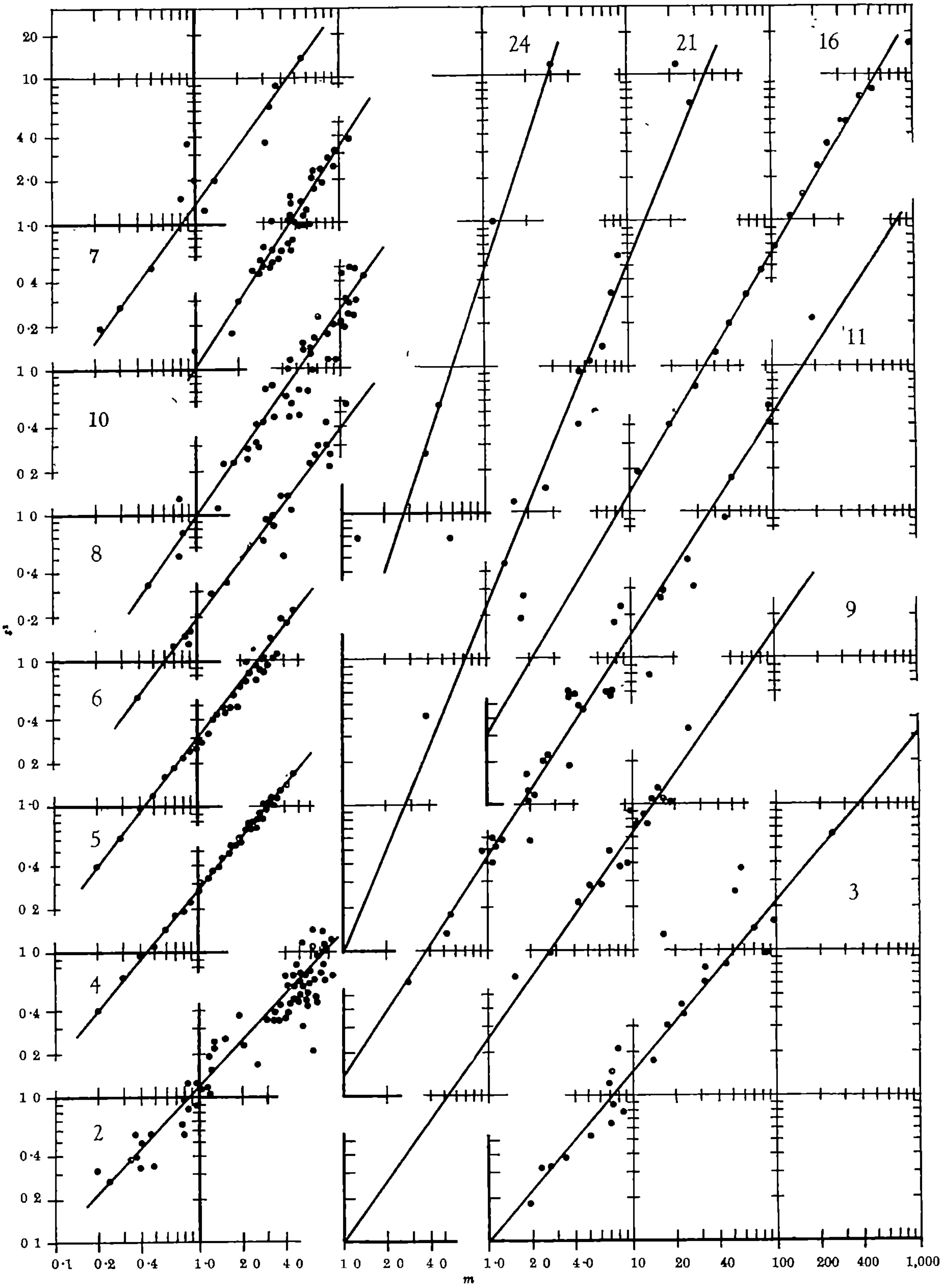

Fig. 1. Variance × mean on log × log scale for material numbered as in Table 1. The axes (heavy lines) are staggered to accommodate as much material as possible

a is evidently largely, possibly wholly, a sampling or computing factor, depending upon the size of sampling unit and on which estimate of variance is used; this was not clearly stated in some of the analyses. b appears to be a true population statistic, an 'index of aggregation' describing an intrinsic property of the organisms concerned and with a continuous graduation from near-regular ($b \rightarrow 0$), through random ($b = 1$) to highly aggregated ($b \rightarrow \infty$).

To use the analysis of variance technique raw data must first be transformed to make s^2 independent of m. Using expression (2), the general transformation:

$$f(x) = Q\int m^{-b/2}\,\mathrm{d}m \qquad (3)$$

where Q is a constant, is obtained. Integration gives a square root transformation when $b = 1$ and, logically, a logarithmic transformation when $b = 2$, as are commonly used; for highly aggregated populations ($b > 2$) it gives a negative fractional power, for example, a reciprocal, as is appropriate to the powerful transformation required.

The power law appears to hold good down to low densities ($m < 1$) in the material examined here. This implies that populations aggregated at high density tend to become regular when density diminishes, and vice versa; the law may break down eventually or, alternatively, perhaps the concept of aggregation ($s^2 > m$) is inappropriate at these low densities. In any event samples consisting mainly of 0's and 1's are likely to need special statistical treatment.

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SURFACE ORIENTATION AND FRICTION OF GRAPHITE, GRAPHITIC CARBON AND NON-GRAPHITIC CARBON

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THE effect, if any, of surface orientation on the friction of various carbons has not yet been clearly established. Indeed, previous reports on the orientation developed by rubbing are conflicting[1-4]. To clarify the position and hence to improve our understanding of the mechanism of the friction of wear of these materials, an attempt has been made to provide fresh evidence, notably by a comparison of the behaviour of a carbon with a very low degree of graphitization[5] with graphite and graphitic carbon These materials have been rubbed unidirectionally and the surface orientation studied by electron diffraction.

When graphite or highly graphitic carbon is rubbed unidirectionally against itself, mild steel or paper, the surface crystallites rapidly become orientated with their (00l) basal planes at a small angle (5–10°) to the surface with the basal plane normals tilted against the direction of motion of the opposing surface, as shown by the electron diffraction pattern of Fig. 1. The deviation of this orientation from the parallel orientation previously reported[1-3] is small, but nevertheless significant, since, as the coefficient of friction is in the range 0 1–0·2 the present texture is consistent with a compression texture with its axis parallel to the resultant of the normal reaction and the friction forces. Similar textures have been recently reported by Porgess and Wilman[6] to be developed by graphite when abraded with emery.

The following factors could contribute to the formation of this texture: (*a*) basal plane slip; (*b*) the plate-like shape of the crystallites; (*c*) preferential removal of crystallites in other orientations.

Concerning the first of these, an electron microscope study has confirmed that, as previously reported

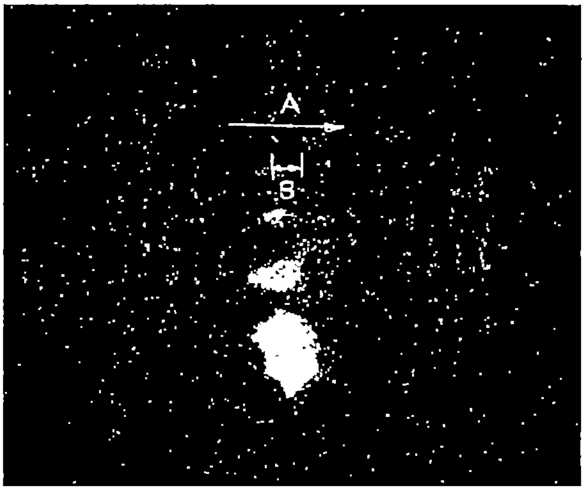

Fig. 1. Electron diffraction pattern of rubbed graphitic carbon. *A*, direction of motion of opposing surface; *B*, basal plane normals make angle of 5–10° to the surface normal

by Williamson[7] and others, basal plane dislocations are present in graphite. Furthermore, we have noted that rubbing gives rise to an extra ring in the electron diffraction patterns which coincides with the normally forbidden (111) ring and this is interpreted as arising from the presence of diffracting crystallites which have been reduced by shear to a few atom planes in the [001] direction[8]. Deacon and Goodman[3] have suggested that in the case of rubbed films of colloidal graphite the plate-like shape of the crystallites plays a decisive part in determining the surface orientation, and we believe that the shape of the crystallites will almost certainly contribute to the observed orientation on massive graphite and graphitic carbon. Finally, it is considered that the development of this preferred orientation is assisted by the preferential removal of crystallites, the basal plane normals of which are tilted in the direction of motion of the opposing surface, for such crystallites will tend to interlock and be plucked out by asperities on the opposing moving surface. The last mechanism, however, could not by itself form the observed orientation on graphite and graphitic carbon, and it is considered that with these materials the first two mechanisms are the most important.

Non-graphitic carbon behaves differently. A surface orientation does develop, but much more slowly than with graphite, and it eventually consists of crystallites the basal plane normals of which are tilted at all angles against the direction of motion of the opposing surface, as shown by the electron diffraction pattern of Fig. 2. This orientation is believed to arise from the preferential removal or wearing away of crystallites with unfavourable orientations. The structure of non-graphitic carbon does not encourage basal plane slip as there is not a unique slip direction, and the crystallite shape does not appear to play a significant part.

Simple compression tests have been employed to verify that non-graphitic carbon does not form a compression texture even when subject to a much higher compressive stress than that necessary to cause graphitic carbon to form the well-known (00*l*) compression texture. This result is in accordance with those of Bacon[9] and Ali, Fitzer and Ragoss[10], and confirms that with non-graphitic carbon the crystallite shape and slip mechanism are likely to be of minor importance in the formation of the rubbing texture.

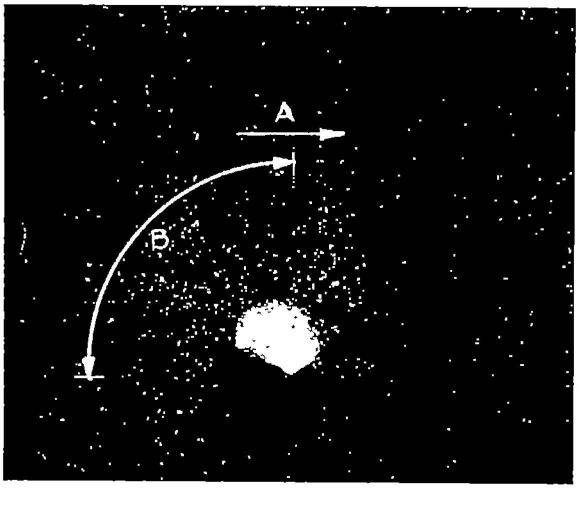

Fig. 2. Electron diffraction pattern of rubbed non-graphitic carbon. *A*, direction of motion of opposing surface; *B*, basal plane normals in this quadrant only

It has been found that the coefficient of friction of non-graphitic carbon is as low as that of graphitic carbon, and yet this low friction is achieved even though a high percentage of the surface crystallites have their basal planes at large angles to the surface. Thus, a parallel orientation is not necessary for the production of a low friction with non-graphitic carbon, and there is no reason to suppose that this is not also true for graphite and graphitic carbon. However, a temporary increase of friction of both graphitic and non-graphitic carbon has been found to occur after reversal of the rubbing direction as shown in Fig. 3 (a similar effect has been mentioned by Fullam and Savage[4]). This increase of friction is considered to arise from the interlocking of those crystallites which are in unfavourable orientations after reversal, as with fibres rubbed against the direction of the scales on their surface (cf. Bowden and Tabor[11]). The mechanism of the friction and wear of graphitic and non-graphitic carbon crystallites with unfavourable orientations is thought to be abrasion rather than intercrystallite attraction.

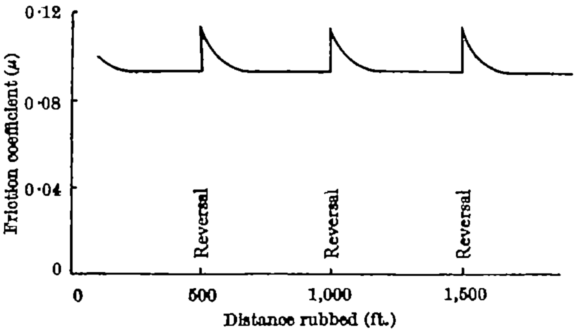

Fig. 3. Increase of friction on reversal of rubbing direction

The present results are interpreted as giving considerable evidence in support of the view that the low friction of graphite and non-graphitic carbon is due to the low adhesion between crystallites[3] rather than to low shear strength[12].

A more detailed account of this work will be published elsewhere.

We are indebted to the Directors of the English Electric Co., Ltd., for permission to publish this communication. We also wish to thank Mr. R. Brew, who took some of the electron diffraction patterns, and Mr. R. Suhr, who supplied X-ray results on the compression textures.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Rotation Period of the Artificial Satellite 1958δ1

RECENTLY, C. R. Faulkner[1] has suggested that changes of the rotation of 1958δ1, especially the decrease of the period during the last days in November 1958, may be explained in part by a strong westerly wind in the upper atmosphere.

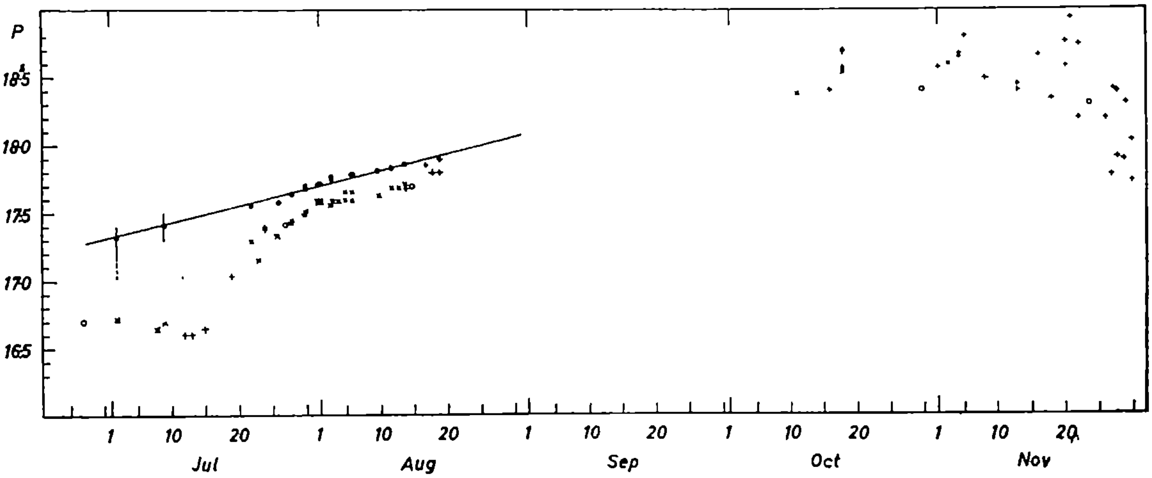

Fig. 1. Period of rotation of 1958δ1. ⍿, Period of rotation with limits of error (October 18 : two possible solutions). ×, Period of scintillation from observations in Babelsberg and Potsdam. ○, Uncertain (November 23 . amplitude of light variation very small). +, Scintillation period from some other sources (ref. 7 and Tatum, J. B., private communication)

Consider first the influence of the rotation of the Earth's atmosphere on the speed of the rotation of the satellite. According to King-Hele[2], in the presence of atmospheric rotation, a factor F, which is 0·95 for 1958δ1, must be added to the aerodynamic force acting in the direction opposite to the velocity of the satellite. Similarly, the formula for the momentum, braking the rotation of the satellite[3], must be altered to the form (neglecting for a moment the component of the atmospheric rotation acting vertically to the path of the satellite):

$$D_B = \text{const.}\, \rho B L^3 \left[v\sqrt{F}\, \omega \mathbf{F}_1 - \frac{1}{3H} v^2 F\, \mathbf{F}_2 \cos \angle B \right] \quad (1)$$

This formula holds for a long cylinder of diameter B and length L ($L \gg B$). $\mathbf{F}_1$ and $\mathbf{F}_2$ are the drag-coefficients for rotation; they change by a maximum of 24 per cent, depending on the position of the rotational axis. H is the scale-height of the atmosphere. $\angle B$ denotes the angle between the pole of rotation and the pole of the orbit of the satellite. The introduction of the factor F in D_B changes this momentum to approximately 0·97 (for 1958δ1) of its former value.

Further, the rotation of the satellite will be influenced by the component of the rotational velocity of the atmosphere, acting vertically to the orbit of the satellite:

$$v_\perp = r_p\, \omega_e \sqrt{(\cos^2\varphi - \cos^2 i)}$$

Here ω_e means the Earth's angular velocity, r_p is the distance from the Earth's centre at perigee, i the inclination of the orbit to the equator. This component is of influence only in the vicinity of the equator ($\varphi = 0$), but even there, its contribution is limited to a maximum of 1·5 per cent of the momentum given by (1), because its action is equivalent to a small displacement (max. ~4°) of the position of the rotational axis with respect to the orbit of the satellite. This causes the coefficients $\mathbf{F}_1$ and $\mathbf{F}_2$ in (1) to change slightly (by maximum of 1·5 per cent as mentioned above).

Because of the smallness of all these factors, we think it impossible, using the observations so far available, to prove that the rotating atmosphere can have an effect on the rotation of satellites, and even less provide evidence of strong winds in the upper atmosphere. From the theoretical point of view, an acceleration of the rotation as indicated in the last days of November (see Fig. 1) is only possible for periods exceeding about 100 sec., provided the position of the rotation axis is favourable.

It is quite certain that the observed period of flashing of 1958δ1 is not identical with its true rotation period[4,5]. An attempt to calculate the real period from the observed flashes has shown[6] that very great deviations up to about 0·9 sec. from the true period may occur, especially if the distance of the satellite from the observer is small (for example, July 1–25, 1958). A similar effect may be present at the end of November. Therefore it is not necessary to explain the behaviour of the rocket at that time by a real acceleration of its rotation. Unfortunately, it was not possible for us to determine the true period from the known observations in November, because of the lack of a sufficient number of exactly known moments of single flashes.

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MY estimate of a decrease of 0·3 sec. in the rotation period (strictly the 'tumbling' period) is the residual effect, after making allowance for the difference between the flash period and the true rotation period Observing from the north of Scotland, I found that by November 27–28, 1958, the flash period had fallen to 8·95 sec. in transits across the south-west at about 0·8°/sec.

I think it is possible to show that only about half the decrease can be attributed to more rapid movement of the observers' line of sight, if it is assumed that the number of flash cycles could never be more than one greater than the actual number of 'tumbles'.

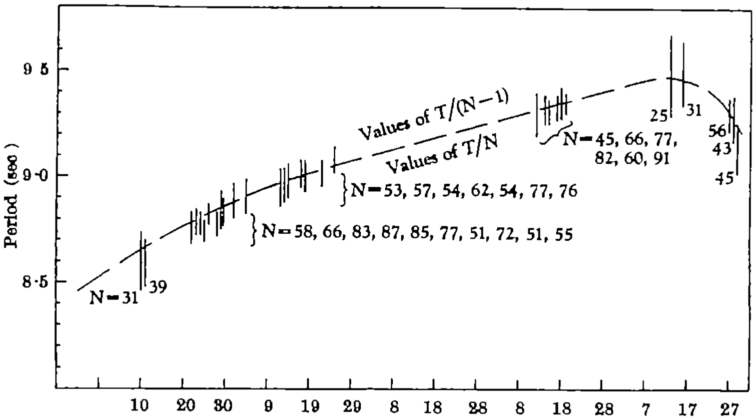

Fig. 1. Tumbling period of satellite $1958\delta 1$, estimated from observations of large numbers of flashes

In constructing Fig. 1, I have used only published observations[1,2] of large numbers of flashes. Each result gives an initial range of uncertainty as drawn between T/N and $T/(N-1)$, where T is the interval containing $(N+1)$ flashes.

The expression for the full rotation period:

$$P = 2T/(N - \theta/180)$$

gives consistent results, where θ is the angle of movement of the line of sight during an observation. The broken curve in Fig. 1 contains what appear to be essential features of the variation. Apart from timing errors, the true curve must pass through the tie-line joining T/N to $T/(N-1)$ for each observation, at a position depending on the value of θ, and therefore closer to $T/(N-1)$ when θ is large.

Any southern hemisphere observations (near apogee) during May or June 1958 would be a valuable check on the form of the early variation.

If it is accepted that a real change took place, at least in November, there follows the problem of finding an explanation. The idea of a 'cross-wind' introduces a non-symmetry of the correct kind, but I agree it should be stated relative to the satellite, and so represent the combined effect of rotation of the atmosphere and any westerly flow greater than the angular velocity of the Earth.

It seems necessary to seek an additional combination of deviations from, and extreme variations within, the ideal model used by P. Notni and H Oleak in their very thorough analysis.

During an interval when the change in angle B is small, the braking moment D_B is controlled by ρ, the air density at perigee. ρ can be calculated from the height of perigee[3], the air density at that height[4], and the deviation from the mean experienced by this satellite[5].

From August until the end of October 1958, the increase of ρ was small, and, apart from the 28-day period of solar disturbances, regular. During this period, the braking moment appears to have been constant. In November, ρ increased at a greatly increasing rate, while my Fig. 1 represents an increasing acceleration of tumbling. The data are not accurate enough to prove that the increase of acceleration is proportional to the increase of air density, but an explanation of the changes can be advanced on this assumption.

This requires proof first of a mechanism to convert most of the air friction to accelerating torque, and second, a main braking force independent of the air density.

The acceleration might arise from large non-symmetries of shape and weight distribution in the rocket body, and a true windmill effect

A precise evaluation of the contribution of magnetic damping may prove to be significant. For the case of a small rapidly rotating spherical satellite, Davis, Whipple and Zirker[6] found that the effect of air friction should be "several orders of magnitude less" than the expected damping of spin by the Earth's magnetic field. For $1958\delta 1$, there are factors of 500 in the rate of spin, and perhaps 10 in the ratio of axial length to diameter.

Note added in proof. I have not yet been able to study the discussion of magnetic retardation by Notni and Oleak in their ref. 6.

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HD 101065—a G0 Star with High Metal Content

On April 26, 1960, in the course of a spectroscopic survey of stars with large proper motions, a spectrum of the eighth magnitude star *HD* 101065 was observed with the Zeiss spectrograph at the Newtonian focus of the 74-in. telescope at Mount Stromlo Observatory. This star, classified as a *B*5 star in the Henry Draper Catalogue, proved to be a much cooler star with a peculiar spectrum of unknown type. The spectral type of this star as estimated from the continuum, and later confirmed by three-colour photometry undertaken at my request by Mr. J. B. Whiteoak, is *K*0. The spectrum, however, shows strong hydrogen lines corresponding to class *F*8 or *G*0. The assumption that this star may have a composite spectrum could not be substantiated by the appearance of the spectrum in the violet region since the strength of all Balmer lines uniformly corresponds to that of a *G*0 star. The constant radial velocity of + 2·3 ± 1·0 km./sec. (based on three plates) and the six-colour photometry described here also suggest that the star *HD* 101065 is not a spectroscopic binary.

In view of the fact that no explanation could be found for the discrepancy between the spectral type determined from the continuum and from hydrogen lines (besides the obvious fact that the star in question has a peculiar spectrum) at my request this star was recently observed photoelectrically by Dr. G. E. Kron in six colours (private communication). The results of these observations are shown in Table 1 together with similar data for *HD* 19373—a normal main sequence *G*0 dwarf, ι Persei[1]—and *HD* 140283—an extreme *G*2 sub-dwarf (Stebbins, J., and Kron, G. E., private communication). Like the two other stars, *HD* 101065 is in all probability a dwarf, as the fairly high annual proper motion of 0·054″ and the six-colour photometry suggest. All three stars agree well in three colours, blue, green and red, and therefore should have essentially the same effective temperature not differing much from that of the Sun. If the difference in the infra-red is interpreted as a temperature effect the star *HD* 101065 should be the hottest and the sub-dwarf *HD* 140283 the coolest, but the difference should not be large. The three stars differ, however, considerably in their violet and ultra-violet colours. The sub-dwarf *HD* 140283, which according to L. H. Aller and J. L. Greenstein[2] and B. Baschek[3] contains only a negligible amount of metals in its atmosphere, possibly only 1/200 of the amount of metals in normal main sequence dwarfs, is the bluest, while the star *HD* 101065 is the reddest. The differences in the violet and ultra-violet are due to the differences in the degree of blanketing, that is, to the differences in the abundance of metals. The star *HD* 101065 should therefore have a much higher abundance of metal in its atmosphere than main sequence dwarfs. This is also suggested by the appearance of its spectrum, which contains about 500 lines, mostly unidentified, in the range λλ3922–4528 Å. on plates with a dispersion of 36 Å./mm. at Hγ, taken with the three-prism spectrograph at the Cassegrain focus of the 30-in. Reynolds telescope.

Table 1

Colour	*U*	*V*	*B*	*G*	*R*	*I*
λ in Å. / Star	3530	4220	4880	5700	7190	10,300
HD 140283	−0·52	−0·27	−0·04	−0·01	+0·03	+0·07
HD 19373	−0·16	−0·12	−0·03	−0·02	+0·05	+0·13
HD 101065	+0·08	+0·06	−0·04	−0·02	+0·07	+0·20

The high abundance of metals is also suggested by the appearance of the continuum. It can be expected that the contribution of metals to the continuous absorption coefficient would suppress the continuum below 4300 Å. or possibly up to λ4400 Å., and therefore a high content of metals would change the continuum in the sense that the star would appear to be of a later type. Since the spectral type estimated from the continuum and from the three-colour photometry is *K*0, while the temperature of the star indicates a spectral type *G*0, the abundance of metals which would cause such a marked change in the appearance of the spectrum must be very high indeed. It is hoped that the spectroscopic study which I have started will provide in the near future a precise figure for the abundance of metals. I expect that the abundance of metals will prove to be many times higher than that of normal main sequence stars.

The energy distribution based on photoelectric spectrophotometry recently secured at my request by Prof. L. H. Aller will be described in another paper now in preparation. This paper will also contain a detailed description of the spectrum; the main features, however, are briefly noted here. The ionized calcium lines *H* and *K*, which are very prominent in normal *F* and *G* dwarfs and even in sub-dwarfs, are faint and approximately equal in strength to hydrogen lines. Also, the absorption spectrum of iron is greatly suppressed and only three lines of iron (λλ4045, 4063, 4071 Å.) can be seen beyond any doubt; the line at λ4045 Å. is fairly strong. The lines of ionized strontium at λ4077 Å. and of barium at λ4554 Å. on the contrary are considerably strengthened in comparison with normal dwarfs. The spectrum shows about 500 lines between λλ3922 and 4528 Å., the majority of which have not yet been identified. Preliminary identifications seem to suggest that many lines should be attributed to rare earths. The spectrum does not show any *G* band, which could be interpreted as an indication that the abundance of carbon is low.

The high abundance of barium and strontium—two elements with the magic number of neutrons in their nuclei—as well as the high abundance of rare earths indicate that the star *HD* 101065 is in an advanced stage of stellar evolution. The star apparently passed through a stage of evolution in which heavy elements were built up by slow neutrons. It presents the same evolutionary problems as *S* stars, complicated in addition by the fact that in all probability it is a dwarf.

I wish to thank Dr. G. E. Kron, who at my request observed the star *HD* 101065 in six colours in spite of the fact that this meant a loss of valuable observing time during his short stay on Mount Stromlo, J. Stebbins and Dr. G. E. Kron for their permission to use unpublished data on *HD* 140283, and Mr. J. B. Whiteoak for securing the *UBV* photometry of *HD* 101065 at a time when this could not be done by me.

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RADIOPHYSICS

The Total Electron Content of the Ionosphere during the Magnetic Disturbance of November 12–13, 1960

MEASUREMENTS of the total electron content of the ionosphere by observation of the differential Faraday fading of lunar radio echoes on two closely spaced frequencies were first made by Evans[1]. Use of a transit aerial limited observations to 1 hr. per day and permitted only the mean diurnal variation of the total electron content (n_t) to be obtained. Similar measurements are now being made with equipment mounted on the 250-ft. Jodrell Bank radio telescope; this allows up to 16 hr. continuous observation a day and gives reliable information on short-term and day-to-day variations in n_t. The frequencies used are 100·60 and 99·40 Mc./s.

The present communication presents some results obtained during a magnetic disturbance during November 12–14, 1960. The sudden commencement preceding the main phase of the disturbance occurred between 12.00 and 15.00 hr. U.T. on November 12, and the most intense phase of the disturbance ($K \geqslant 7$) was between 18.00 hr. on November 12 and 15.00 hr. on November 13. Curves of n_t vs. time for each of the days November 11–14 are shown in Fig. 1 (full line). The periods covered are those when the Moon was at a high enough elevation to permit useful observations. The curves have been corrected to local mean time at the sub-ionospheric point, and the mean sub-ionospheric latitude is 50·0° N. Also shown in Fig. 1 are hourly values of the maximum electron density N_m (points) derived from f_oF2 values for Slough (51·5° N. geographic, 54·3° N. geomagnetic) and 3-hourly K indices for the Hartland Magnetic Observatory.

K was low throughout the observational periods on November 11 and 12, and the n_t curves for these two days are normal. The disturbance began shortly after moonset on November 12, and the n_t curve for November 13 covers a large part of the most intense phase of the disturbance. n_t is lower than normal during the pre-sunrise period, and also shows an abnormally low rate of increase after sunrise, so that its midday value is less than one-third of that on the two preceding days. N_m is unmeasurable at Slough from 19.00 on November 12 until 03.00 on November 13, and is abnormally low throughout November 13. Both n_t and N_m are very low in the pre-sunrise hours of November 14 and both have returned to their normal values by midday on this day. Aurora was visible almost overhead at Jodrell Bank shortly before moonrise on November 13, but had retreated to the northern horizon before observations began. Visual observations of auroræ in the British Isles for the night of November 12–13 (Paton, J., private communication) show that it is unlikely that an aurora was present in the line of sight to the Moon at any subsequent time.

Table 1. BEHAVIOUR OF CERTAIN PARAMETERS AT SELECTED TIME DURING NOVEMBER 11–14, 1960

	Nov. 11	Nov. 12	Nov. 13	Nov. 14
n_t at 05.00 L.M.T.* (electrons/cm.^{2} × 10^{-12})	7·6 ± 1·3	10·9 ± 1·3	2·8 ± 2·1	0·8 ± 0·5
n_t at 10.00 L.M.T. (electrons/cm.^{2} × 10^{-12})	33·8 ± 1·7	35·4 ± 1·7	9·6 ± 2·9	30·2 ± 1·4
dn_t/dt from 07.00 to 10.00 L.M.T. (electrons/cm.^{2}/hr. × 10^{-12})	8·3	8·0	1·9	8·5
n_t/N_m at 05.00 L.M.T. (km.)	450 ± 80	570 ± 70	500 ± 300	200 ± 120
n_t/N_m at 10.00 L.M.T.	200 ± 10	200 ± 10	> 640	220 ± 10

* Local mean time.

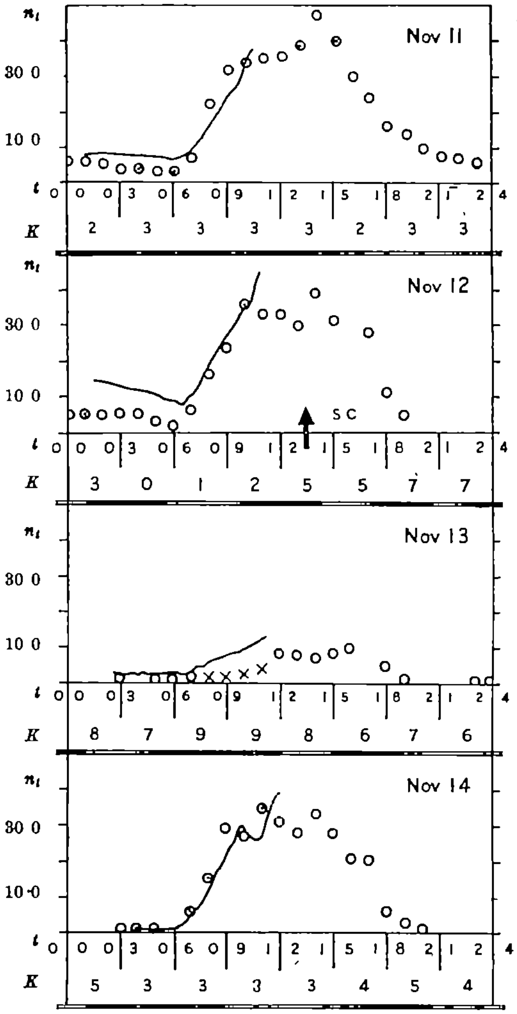

Fig. 1. Total electron content (n_t, full line) and Slough maximum electron density (N_m points) vs. time for each day during November 11–14, 1960. Also shown are Hartland K indices (K) and the approximate time of the sudden commencement on November 12 (S.C.). Upper limits are indicated as ×. Units: n_t, electrons/cm.^{2} column × 10^{-12}; N_m, electrons/cm.^{3} × 10^{-6}; t, hr.

Some relevant parameters for each of the four days are shown in Table 1. Listed here are n_t at 05.00 and 10.00 hr.; the mean rate of increase, dn_t/dt, between 07.00 and 10.00 hr.; and the equivalent column height, n_t/N_m, at 05.00 and 10.00 hr. The error in n_t is proportional to the error of measurement of the total number of Faraday rotations. This latter error is normally ± ½ rotation, and the corresponding errors in n_t are listed in Table 1. The errors shown for November 13 are rather larger than normal. This is partly because the Faraday fading on this day was slow and erratic throughout, and also because the record was partially obscured by interference for about an hour just before dawn.

The pre-dawn values of n_t are very low on both November 14 and 13; since magnetic activity was much lower at this period on November 14 than 13, the low value on the former date can probably be attributed to normal night-time decay following a low noon maximum on November 13. The low value of dn_t/dt after dawn on November 13 suggests that one of the effects of the disturbance may be either an increased rate of recombination or a decreased rate of production of electrons in the lower levels of the ionosphere, where most of the ionization originates. The equivalent column heights at 05.00 hr. lie in the normal range on November 13 and November 14, but this parameter appears to be abnormally high at 10.00 hr. on November 13.

Several workers have measured n_t during magnetically disturbed periods by observations of transmissions of satellites. Hame and Stuart[2] report decreases in n_t at two periods of high magnetic activity near the vernal equinox. Ross[3] finds an inverse correlation between n_t and K_p, the correlation being good in the summer but poor in the winter. Garriott[4] finds normal values of n_t and abnormally high equivalent column heights on disturbed days, the observations applying mainly to the winter half of the year.

These results are not necessarily inconsistent with the present ones, since all the previous observations were made at appreciably lower geomagnetic latitudes than apply here. Maeda and Sato[5] have shown that, at high latitudes, ionospheric disturbances are mainly 'negative' in type (f_0F2 decreases) at all times of the year, while in medium latitudes disturbances are predominantly negative in summer, but may be either positive or negative in winter. The importance of the observations described in this communication is to show that, at least on the present occasion, the reduction in N_m was accompanied by a proportional reduction in n_t. Thus there appears to have been a scaling down of the whole ionosphere rather than a vertical redistribution of ionization within it.

I wish to thank Sir Bernard Lovell for the use of the facilities of the Nuffield Radio Astronomy Laboratories and Dr. J. H. Thomson for valuable guidance. Thanks are also due to the Director of the Radio Research Station (Department of Scientific and Industrial Research), Slough, and the Superintendent of the Magnetic Department, Royal Greenwich Observatory, for permission to quote pre-publication values of f_0F2 and K indices respectively. This work was carried out under U.S. Air Force Contract No. *AF*61(052)–172.

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PHYSICS

Optical Demonstration of Bragg's Law

A simple and rather striking demonstration of Bragg's Law can be given by making use of Lippmann's method for producing colour photographs[1]. In this method a photographic plate with an extremely fine-grained emulsion is placed with its sensitive layer on a mercury surface. It is then exposed from above, through the glass plate, to monochromatic light, for example from a sodium arc lamp. Due to reflexion at the mercury surface, standing light waves are produced in the sensitive layer, which, after development, give rise to parallel equidistant layers of photolytic silver, with a spacing equal to half the wave-length of sodium light.

Such a plate, of dimensions, for example, 3 cm. × 3 cm. (prepared in this Laboratory by B. Phielix, using, with some modification, a method described by H. Lehmann[2]), is fixed on a revolving holder, and inserted in an ordinary spherical glass flask as used in chemical experiments (diameter about 10 cm.). The flask is filled with benzene, to prevent reflexion at the outer surface of the emulsion layer. If now a parallel beam of white light, after passing through the solution, is reflected by the Lippmann plate, the latter acts as an 'interference mirror', and reflects a coloured beam, the colour changing with the angle of incidence. The reflected beam, when falling on a transparent screen, is of sufficient strength to be visible in a lecture room. In the experiment as I have performed it, the colour changes from yellow at a glancing angle θ in the neighbourhood of 90° to violet at θ about 45°.

The arrangement may serve to illustrate the influence of a slight change in spacing on the reflected wave-length. By breathing on the sensitive layer, a slight swelling of the gelatine takes place, which produces a considerable change in colour, from yellow to red for θ close to 90°, whereas for $\theta \sim 45°$ the colour of the reflected beam changes only from violet to blue. Thus also the different sensitivity of 'high-angle' and 'low-angle' reflexions for a slight change in spacing can be illustrated.

I do not know whether this 'application' of Lippmann's plates has already been described elsewhere. In a most interesting paper on "The Crystal Structure of *Tipula* Iridescent Virus as determined by Bragg-Reflection of Visible Light"[3], A. Klug, (the late) Rosalind E. Franklin and S. P. F. Humphreys-Owen analysed a crystal with lattice spacings so large that these could be measured by reflexion of visible light. Dr. Klug told me that cells containing these virus crystals had been used to demonstrate Bragg's law optically. This suggested to me that the above demonstration might be of some interest.

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Acceleration of Deuterons in a Synchrotron

Because of the theoretical interest in the study of the collisions between nucleons, all the large heavy-particle synchrotrons at present operating have been designed to accelerate protons[1]. In many of these machines, however, the frequency of the accelerating voltage can be pre-set to follow any suitable law of variation with magnetic field. There is nothing then to prevent the acceleration to an appropriate energy of particles with an e/m different from that of the proton providing that they can be injected.

In the Birmingham proton synchrotron[2] the radio-frequency law was originally obtained by a rotating condenser which varied the capacity in an oscillator circuit[3]. This system has now been successfully replaced by an analogue computer and a function generator, driven by a signal proportional to the rate of change of magnetic field, the output signal controlling a variable capacitance diode in an oscillator circuit. This allows considerably greater flexibility. The low injection energy and small aperture of the Birmingham machine impose severe limits on the permissible errors of the radio-frequency at injection. As a result, the system requires a signal to noise (and drift) ratio of $10^5:1$, and this has been achieved. It has been found possible to accelerate deuterons readily with this installation.

Deuterium gas was supplied to the radio-frequency ion source of the synchrotron injector. With the same injection energy as for protons (460 keV.) a beam was found to enter the synchrotron vacuum system and to circulate before acceleration at a magnetic field of $\sqrt{2}$ times the value for protons. This beam was carried to full energy (estimated from the magnetic field to be 642 ± 12 MeV. for mass 2) by suitable adjustment of the radio-frequency function generator and was found to circulate at maximum field with a frequency 0·76 times that for protons. The beam was extracted from the synchrotron by the method normally used for protons[4] and was found to have an intensity of 10^8 particles per pulse over an area of 12 cm.^{2} at a distance of 16 m. from the extractor. The range of the beam in copper was measured to be 167 ± 3 gm. cm.$^{-2}$ which, allowing for 7-MeV. energy-loss in polythene and air in the beam path, corresponds to an energy of 640 ± 5 MeV., confirming that the particles accelerated were deuterons. No beam was accelerated when hydrogen gas was fed to the ion source with the radio-frequency generator set for deuterons.

The identity of the accelerated particles was also confirmed in a preliminary experiment to measure the differential cross-section for the elastic scattering of deuterons by protons. Coincidences between scattered deuterons and recoil protons from a hydrogenous target were recorded at the correct correlation angle; when the counter telescopes were moved off this angle the counting-rate dropped to 20 per cent of that previously obtained. It is hoped to continue these observations and to extend them to a study of the deuteron–deuteron interaction at the available energy.

The new radio-frequency installation was developed from a system proposed by W. Boyd and his team at the National Institute for Research in Nuclear Science, Harwell. We wish to acknowledge the valuable help we have received from them at all stages of the work. We are also indebted to D. A. Gray of the Institute for assistance in the problem of deriving signals from the rising magnetic field. Many members of the synchrotron group of this laboratory also contributed to this project, in particular K. R. Chapman, who made the necessary adjustments to the ion source, and the Counter Group, who made the range and scattering measurements.

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The Quasi-Longitudinal Approximation to the Appleton–Hartree Equation

IF an electromagnetic wave passes through a medium containing free electrons, which may make collisions with heavy particles, in the presence of a uniform magnetic field, the refractive index and absorption coefficient of the medium are given by the Appleton–Hartree equation[1]:

$$n^2 = (\mu - i\chi)^2 =$$
$$1 - \frac{X}{1 - iZ - Y_T^2/2(1 - X - iZ) \pm [Y_T^4/4(1 - X - iZ)^2 + Y_L^2]^{1/2}} \qquad (1)$$

where μ = refractive index; χ = absorption coefficient; $X = f_0^2/f^2$; $Y_T = (f_H/f)\sin\theta$; $Y_L = (f_H/f)\cos\theta$; $Z = \nu/2\pi f$; f = frequency of wave; f_0 = plasma frequency; f_H = gyrofrequency; ν = frequency of collisions between electrons and neutral molecules; θ = angle between wave normal and magnetic field.

Because of the complicated nature of the above equation the quasi-longitudinal and quasi-transverse approximations, first discussed by Booker[2], are often used.

The quasi-longitudinal approximation holds when:

$$Y_T^4/4Y_L^2 \ll (1 - X)^2 + Z^2 \qquad (2)$$

It is generally quoted as:

$$n^2 = 1 - X/(1 - iZ \pm |Y_L|) \qquad (3)$$

Equation (3) means that the wave behaves very much as if it is being propagated along the direction of the magnetic field, since, for longitudinal propagation, equation (1) reduces to:

$$n^2 = 1 - X/(1 - iZ \pm Y) \qquad (4)$$

However, equation (3) is incorrect, as can easily be seen by considering the denominator of the second term of equation (1):

$$1 - iZ - Y_T^2/2(1 - X - iZ) \pm$$
$$[Y_T^4/4(1 - X - iZ)^2 + Y_L^2]^{\frac{1}{2}}$$

When the inequality (2) holds, this becomes:

$$1 - iZ - Y_T^2/2(1 - X - iZ) \pm |Y_L|$$

and $Y_T^2/2(1 - X - iZ)$ is not necessarily negligible compared with Y_L. In fact, if we assume the usual numerical condition that the inequality (2) is satisfied if the larger quantity is nine times the smaller, then $|Y_T^2/2(1 - X - iZ)|$ may be as large as $\frac{1}{3}Y_L$. The correct approximation is thus:

$$n^2 = 1 - \frac{X}{1 - iZ - Y_T^2/2(1 - X - iZ) \pm Y_L} \qquad (5)$$

and the wave does not behave as if it were propagated along the direction of the magnetic field. It will only behave thus when the term $Y_T^2/2(1 - X - iZ)$ can be neglected, that is, when:

$$Y_T^2/2Y_L \ll |(1 - X) - iZ| \qquad (6)$$

Outside these limits, but where inequality (2) still holds, equation (5) should be used. A similar correction should also be made to the expressions for the polarization.

I am grateful to Prof. J. A. Gledhill for some helpful discussion.

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MINERALOGY

Kilchoanite, a Polymorph of Rankinite

THE compound $9CaO6SiO_2H_2O$ was originally synthesized and described by Roy[1,2], who called it phase *Z*. Revised data on its properties have been given by Roy, Gard, Nicol and Taylor[3], who showed that, when hydrothermally synthesized at 715° C. and with a pressure of 1,100 bars, its composition was $3CaO2SiO_2$ and that it became inverted to rankinite on heating between 954° C. and 1,090° C.

A mineral corresponding to phase *Z* has been found in limestones thermally metamorphosed by gabbro at Ardnamurchan, Scotland, and Carlingford, Eire. It has been named kilchoanite after the village in Ardnamurchan near where it was first found.

It has the following properties: colourless, slightly turbid due to minute inclusions and no visible cleavage, α 1·647, γ 1·650, $\gamma - \alpha$ 0·003, $+ 2V$ 60°, dispersion distinct $r > v$, density 2·992. In thin section the interference colours are commonly weak ultra-blue and ultra-brown.

Kilchoanite has always been found as a replacement of rankinite and no crystal form has been observed. Single crystal X-ray data indicate that it is orthorhombic with *a* 11·42 Å., *b* 5·09 Å., *c* 21·95 Å. (all ± 0·05 Å.). The Laue group is *mmm* and the probable space group *I mam* or *I ma*2. X-ray powder data, given in Table 1, agree with those of Roy[2] for the phase *Z* apart from discrepancies which could be due to impurities in the latter.

Separation of kilchoanite for analysis was difficult owing to the complexity of the mineral assemblages with which it is associated and because of its irregular distribution. A rock containing no rankinite was eventually used to prepare a sample in which spurrite was the only impurity. Having established that kilchoanite contained no carbon dioxide, this sample was analysed. On the assumption that all the carbon dioxide was in the contaminating spurrite, this analysis, together with the cell dimensions and the determined density, gave the calculated cell contents as:

$$(\mathrm{Ca}_{23\cdot47}\mathrm{Mn}_{0\cdot03}\mathrm{Fe^{III}}_{0\cdot05})_{23\cdot55}(\mathrm{Si}_{15\cdot71}\mathrm{Al}_{0\cdot35})_{16\cdot06}(\mathrm{O}_{55\cdot53}\mathrm{OH}_{0\cdot47})_{56\cdot00}$$

This value is in close agreement with $8(Ca_3Si_2O_7)$ and confirms that kilchoanite corresponds to the phase *Z* and is a polymorph of rankinite. Further confirmation is found in that on heating at 1,000° C. for 10 hr. a single crystal of kilchoanite became inverted to a polycrystalline aggregate of rankinite.

The metamorphic assemblages from which kilchoanite is developed consist essentially of:

rankinite—spurrite—melilite
rankinite—wollastonite—melilite

These give way to assemblages such as:

rankinite → kilchoanite — spurrite → tilleyite — melilite — cuspidine
kilchoanite — tilleyite — melilite — cuspidine
kilchoanite — spurrite — grossular — idocrase — cuspidine
kilchoanite — wollastonite — grossular — idocrase

It is suggested that kilchoanite does not form in normal prograde metamorphism as under conditions of relatively high carbon dioxide pressure the reaction spurrite + wollastonite → rankinite + carbon dioxide, is above the stability limit of kilchoanite. If, however, the rock has been decarbonated, a later retrograde phase or repeated thermal metamorphism under a low carbon dioxide pressure may convert the rankinite to kilchoanite at lower temperatures in the presence of water.

Further work on this mineral and these rocks is in progress and will be reported in detail later.

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Table 1

Kilchoanite		Phase *Z*				Kilchoanite		Phase *Z*			
d_{hkl}	*I*	d_{hkl}	*I*	$d_{\text{calc.}}$	*hkl*	d_{hkl}	*I*	d_{hkl}	*I*	$d_{\text{calc.}}$	*hkl*
5·17 Å.	*m*	5·10 Å.	0·2	5·07 Å.	202	2·26	*vw*	2·26	0·05	2·27	222
—	—	4·76	0·1	—	—	2·18	*vw*	—	—	—	—
4·26	*w*	—	—	4·28	112	2·06	*w*	—	—	—	—
—	—	4·18	0·1	4·18	013	1·996	*w*	—	—	—	—
4·00	*w*	3·97	0·2	3·97	204	1·964	*ms*	1·964	0·3	—	—
—	—	3·76	0·1	3·74	211	1·906	*m*	1·902	0·15	—	—
—	—	3·67	0·1	3·66	006	1·872	*m*	—	—	—	—
3·56	*ms*	3·56	0·4	3·54	114	—	—	1·833	0·3	—	—
3·39	*vw*	—	—	3·37	213	1·814	*w*(*b*)	—	—	—	—
3·07	s_3	3·06	0·75	3·08	206	1·779	*w*	—	—	—	—
				3·05	310	1·745	*m*	1·749	0·2	—	—
2·89	s_1	2·88	1·0	2·88	116	1·694	*m*	1·692	0·1	—	—
				2·87	215	1·659	*vw*	—	—	—	—
—	—	2·84	0·3	2·86	400	1·602	*w*	—	—	—	—
2·77	*vw*	—	—	2·76	402	1·569	*w*	1·570	0·05	—	—
—	—	2·73	0·1	2·74	008	1·559	*w*	—	—	—	—
2·68	s_2	2·67	0·75	2·69	017	1·548	*w*	—	—	—	—
				2·67	314	1·526	*m*	—	—	—	—
2·55	*m*	2·55	0·2	2·55	020	1·487	*w*	1·489	0·05	—	—
2·48	*m*	2·48	0·2	2·48	121	1·418	*m*	—	—	—	—
				2·48	411	1·398	*vw*	—	—	—	—
2·42	*m*	2·42	0·3	2·43	217	1·379	*vw*	—	—	—	—
2·36	*ms*	2·35	0·15	2·36	118	1·357	*vw*	—	—	—	—
				2·35	123	1·337	*vw*	—	—	—	—
—	—	—	—	2·34	316	1·332	*w*	—	—	—	—
								etc.			

CRYSTALLOGRAPHY

Etch Patterns on Cadmium Crystals grown from Vapour

SERVI[1] showed complex spirals on the basal plane of cleaved zinc crystals after etching, and spiral terraced etch pits have been obtained on zinc crystals[2] grown from vapour using 'Superoxol' etch. Presented here are two types of etch figures obtained on 99·9 per cent cadmium crystals grown from vapour. Of several etching reagents that had been tried the most suitable one was a saturated solution of picric acid in acetone and a normal time of 2 min. was decided on. For longer periods of etching the solution acted as polishing agent.

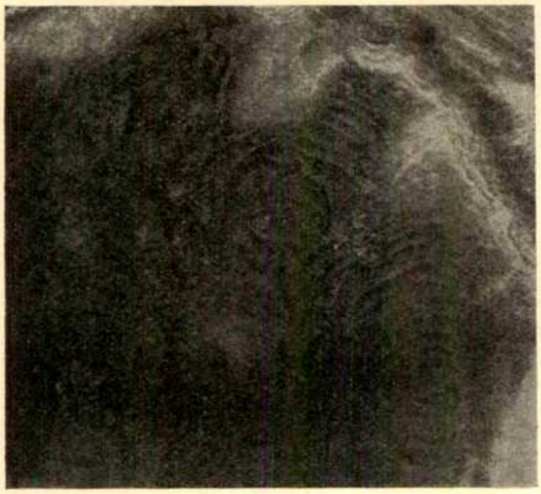

Fig. 1

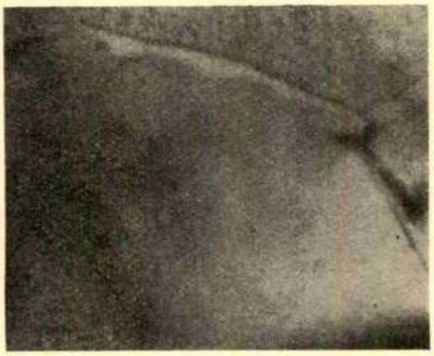

Fig. 2

Several complex spirals were observed on the basal planes of some of the crystals after etching (Fig. 1). The hexagonal symmetry of the basal plane is clearly indicated in these spirals. This suggests that crystal symmetry is a factor in etch spiral formation. As the spiral moves outwards it breaks up into terraces, and a sort of interlacing occurs. It is interesting to note that the spirals break and make along crystallographic directions. In some cases etch patterns closely resembling hexagonal spirals with the dislocation line emerging at the centre were observed (Fig. 2). It is evident that these etch pits are formed at the sites of dislocations.

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GEOLOGY

Palæoecology of Carboniferous Peat Bogs

THE microscopic examination of coals can provide valuable information on their mode of formation. The works of Teichmüller[1] and Thomson[2] on the Rhineland brown coal are notable examples. Apart from the investigations of Kremp[3] and Smith[4], little of the published work on the possible origin of the different petrographic types of coal takes account of the evidence provided by the plant spores in coals of Carboniferous age.

Recently, a systematic study of the petrology and palynology of British bituminous coals from the Lower and Middle Coal Measures of the East Pennine coalfield has yielded results which are not only of theoretical importance, but also provide information which will permit the more effective use of spores in the correlation of coal seams. These results are being prepared for publication.

Broadly speaking, each of the more important petrographic coal-types possesses a distinctive assemblage of miospores (and probably megaspores). Four main types of assemblage have been recognized, associated respectively with predominantly bright, interbanded, and the two distinct petrographic types of dull, coal. These assemblages are related in the order in which they succeed one another in seam profiles. A full sequence is found only in seams containing dull coal characterized by the petrographic constituents micrinite (massive) and exinite, the latter largely comprising the exines of a single spore *Densosporites sphaerotriangularis* Kos. A sequence may be repeated more than once within a seam profile.

It is possible to recognize, within the profiles, horizons with distinctive petrological and palynological features which include allochthonous elements. These horizons probably mark the flooding of the bog surface and their occurrence may modify the development of a sequence.

From what is known of the botanical affinities of the spores, it is possible to visualize something of the nature of the plant succession corresponding to the sequence of miospore assemblages. The conditions under which peat formed may be inferred from the nature and possible origin of the petrographic constituents associated with the different assemblages of spores.

Any hypothesis as to the conditions under which peat was formed in the Carboniferous must take account of the evidence provided by work on the frequency and stratigraphic ranges of spores found in the seams in all the major British coalfields. These have demonstrated an essentially identical pattern of distribution and abundance in the different coalfields. Also to be considered is the occurrence, over wide areas within a coalfield, of beds, often nearly constant in thickness, of dull coal containing massive micrinite and spores predominantly of one type. This petrographic type is known from coals of paralic and limnic origin, and is a conspicuous feature of the middle and upper portions of certain seams in the Lower and Middle Coal Measures in Britain.

With regard to the palynological evidence, it is thought unlikely that the quantitative and qualitative composition of the miospore assemblages from whole seams of coal deposited contemporaneously would show common features if depth of water cover or salinity had been the controlling factor determining the nature of the vegetation growing in the areas of peat deposition. Considering those features of the dull coal to which reference has been made, W. G. Chaloner (personal communication) suggested that the formation of peat of a distinctive type within certain Carboniferous bogs may have taken place under conditions similar to those which give rise to blanket bogs at the present time, and that the causative factor was more likely to be a climatic (for example, rainfall) fluctuation than simply one of water-level.

Such a mode of origin accords well with the observed facts. By analogy with present-day raised bogs, it seems reasonable to suggest that the vertical changes which took place as peat accumulated in these Carboniferous deposits may have been the result of the increase in acidity and the decrease in nutrients available for plant growth. On this view, therefore, the sequences which have been observed may mark the change from peat of eutrophic to that of oligotrophic type.

It is of interest that the study of the peat deposits of north-west Borneo by Andrews and Richards[5] suggests that these peats are of raised-bog type. The vegetation growing on these peats is arboreal and is reported to exhibit a concentric zonation. Profiles from the central parts of the deposit show a succession of peat types formed from the same plant communities that occur at the present time from the periphery to the centre. In this and other respects there is probably a close analogy with the structure observed in certain coals which originated in Carboniferous peat bogs.

The mode of peat formation, which it is suggested might account for the petro-palynological characteristics of certain Carboniferous coal seams, is different from that postulated by Teichmuller[6] for the brown coals of the Rhineland.

The possible climatic control of bog structure in the Carboniferous would explain features of the distribution and abundance of miospores in coal seams of the period.

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CHEMISTRY

Polymers and the Theory of Numbers: Molecular Weight Distributions from Rheological Measurements

Recently, V. S. Nanda and R. K. Pathria have shown how certain problems from the theory of numbers may be applied to the theory of polymer degradation[1]. Their work appears to be one of the very few applications of the theory of numbers to physical or chemical problems. We wish to report a new application of another classical theorem of the theory of numbers to yet another problem in the field of polymer physical chemistry. By the use of this theorem, it appears to be possible to obtain the molecular weight distribution of a polymer sample from rheological measurements.

Rouse's equation[2] for the real part of the complex viscosity of a polymer solution may be written as:

$$\eta_1(\omega) = K \sum_{p=1}^{\infty} f(\omega/p^2) \qquad (1)$$

where η_1 is the real part of the complex viscosity, ω is the frequency and K is a frequency-independent parameter.

The function $f(\omega/p^2)$ is a type of Stieltjes transform of the molecular weight distribution function, $Z(M)$, defined as $MW(M)/\overline{Mw}$, where $W(M)$ is the weight fraction of polymer between molecular weights M and $M + dM$, and $\overline{Mw}$ is the weight-average molecular weight. In fact, $Z(M)$ is related to $f(\omega) \equiv f(\omega/1^2)$ by the relation:

$$Z(M) = \mathscr{S}\{(f(\omega)\} \qquad (2)$$

where $\mathscr{S}$ stands for inversion of the Stieltjes transform. Such an inversion may be obtained to any desired approximation through the use of the inversion formula of Hirschman and Widder[3]. In order to obtain $f(\omega)$ from equation (1), we use the classical number-theory theorem of Moebius[4] to obtain:

$$f(\omega) = K^{-1} \sum_{s=1}^{\infty} \frac{\mu_s}{s^2}\eta_1(\omega/s^2) \qquad (3)$$

where μ_s is the Moebius μ-function defined as equal to $+1$ if s is $+1$ or if s can be factorized into an even number of unlike primes and -1 if s can be factorized into an odd number of unlike primes, and zero for other values of s. Combining (2) and (3), we have:

$$Z(M) = \mathscr{S}\left\{K^{-1} \sum_{s=1}^{\infty} \frac{\mu_s}{s^2}\eta_1(\omega/s^2)\right\} \qquad (4)$$

Thus if $\eta_1(\omega)$ is known experimentally for any polymer, equation (4) gives an equation for directly obtaining the molecular weight distribution function. The potential usefulness of such a relation lies in the fact that it may present a far easier and more rapid method of experimentally determining the molecular weight distribution of a polymer than has heretofore been possible.

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The BH_4^- Ion in the Lithium Borohydride Lattice

Price[1] reports an anomalous band at about 1,250 cm.$^{-1}$ in the paraffin-oil mull spectrum of lithium borohydride at room temperature. This band does not occur in the room-temperature spectra of sodium borohydride[2,3] and potassium borohydride[3] or in the low-temperature spectra of sodium borohydride[2,4] and potassium borohydride (Schutte, C J. H., unpublished results).

The crystal structure of lithium borohydride is orthorhombic (space-group V_h^{16}) with four molecules per unit cell[5].

V_h^{16} has the following sites[6]: $2C_i(4)$ and $C_s(4)$. A comparison of the site groups of the space group and the sub-groups of the ionic point group (T_d) shows that the BH_4^- ion is on site C_s. The local-symmetry group of the ion, constructed from the common elements of the site group and the space group, is C_s.

The symmetry of the perturbing crystal field acting on the tetrahedral BH_4^- is thus C_s. Under the action of a perturbing field of symmetry C_s the vibrational levels of the tetrahedral ion transform as follows :

$$\begin{tikzcd}[row sep=small]
T_d & C_s \\
A \arrow[r] & A' \\
 & A' \\
E \arrow[ur] \arrow[dr] & \\
 & A'' \\
 & A'' \\
F_2 \arrow[ur] \arrow[r] \arrow[dr] & A' \\
 & A'
\end{tikzcd}$$

that is, the degeneracy of all the levels is removed and all the levels are infra-red active (only the F_2 levels are infra-red active under T_d).

This means that all four tetrahedral fundamentals should be observable in the infra-red absorption spectrum, as well as all the overtones and combination bands In addition, all the degenerate bands should show fine structure caused by the removal of all the degeneracy by the perturbing crystal field.

The splitting of the degenerate bands would probably be obscured by the broadness of the bands at room temperature, but ν_1 and ν_2 should be observable. Unfortunately, the band due to the ν_1 vibration lies among ν_3 and its satellite bands (at about 2,300 cm.$^{-1}$) and could easily be obscured by these.

From the spectrum published by Price[1] it can be deduced that ν_2 should occur around 1,280 cm.$^{-1}$ (from the frequencies of ν_4 and $\nu_2 + \nu_4$).

The anomalous band reported by Price[1] at about 1,250 cm.$^{-1}$ in the spectrum of lithium borohydride can thus probably be assigned to the ν_2 vibration of the BH_4^- ion in the lithium borohydride lattice. This band is normally inactive in the infra-red, but occurs here because of the 'reduction' of the symmetry of the ion by the crystalline field, which is of the type C_s.

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Isolation of D-Glycero-D-galacto-heptitol from the Wound Exudate of Avocado Trees

D-GLYCERO-D-GALACTO-HEPTITOL has been isolated from the semi-crystalline exudate of wounds in the bark of *Persea americana* Mill.

The crude material was a pale brown solid which did not reduce Fehling's solution, and gave evidence of only one major component on paper chromatograms. Two recrystallizations of this solid from aqueous methanol afforded a colourless product in the form of needles which had a melting-point of 183–185°, no depression on admixture with authentic perseitol.

A portion of the crude exudate was acetylated with pyridine and acetic anhydride to give a crystalline acetate, which after recrystallization from aqueous ethanol had a melting-point of 119–120·5°, no depression on admixture with authentic perseitol heptacetate. This derivative also had $[\alpha]_D^{22°} -14°$ (about 2·02 in chloroform); literature value[1] $[\alpha]_D^{16°} - 13·3°$ (chloroform).

A further portion of the crude material was treated with benzaldehyde and acid according to Hudson *et al.*[2]. From this reaction mixture a crystalline derivative was isolated, which after one recrystallization from pyridine–ethanol had a melting-point of 276–278° and $[\alpha]_D^{17°} -62°$ (about 1·95 in pyridine). Hudson *et al.*[2] give values of 277 ± 2° for the melting-point and $[\alpha]_D^{16°} -58·1°$ (about 0·9 in pyridine) for 1,3 : 5,7-di-O-benzylidene-D-glycero-D-galacto-heptitol.

From these results we have concluded that the chief constituent of *Persea americana* Mill wound exudate is D-glycero-D-galacto-heptitol (perseitol), a compound known to be present in the fruit[3] and leaves[4] of this tree.

Thanks are due to Drs. L. W. Klotz and G. A. Zentmyer of the Citrus Experiment Station, Riverside, California, who supplied the exudate described here.

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Monosaccharide Sequence in *Pneumococcus* Type XIV Polysaccharide

PREVIOUS work[1] has established that in *Pneumococcus* type XIV polysaccharide there are two non-reducing end groups of D-galactopyranose, one D-galactopyranose residue linked 1 : 3, one D-glucose residue linked 1 : 4 and two 1 : 4 : 6-linked 2-acetamido 2-deoxy D-glucose residues for every six monosaccharide units. Although the majority of these linkages are β-, some α-D-galactose units may be present. The two major disaccharides left after treatment of the type XIV polysaccharide with *N* hydrochloric acid at 100° for 2½ hr. were 4-O-β-D-glucosyl 2-acetamido 2-deoxy D-glucose and 3-O-β-(2-acetamido 2-deoxy)-D-glucosyl D-galactose. Less drastic hydrolysis has enabled other disaccharides to be isolated and the structure of these is the subject of the present communication.

The polysaccharide (3·606 gm.) was hydrolysed with 2 *N* sulphuric acid (430 ml.) at 85–90° C. for 2½ hr. After neutralization with barium carbonate, the hydrolysate was passed through 'Amberlite *IR*' 120 (H+), which was eluted with water to yield neutral sugars (1·8 gm.) and with 2 *N* sulphuric acid to remove basic sugars (1·0 gm.). Fractionation of the neutral sugars on a charcoal–'Celite' column[2] gave a D-galactose–D-glucose mixture (about 1·4 gm.), N-acetylglucosamine (40 mgm.), disaccharides I and II (4 mgm.), III (11 mgm.), V (30 mgm.), VI (3 mgm.), trisaccharides IV and V (30 mgm.), as well as trisaccharide VI and another oligosaccharide (30 mgm.).

The basic sugars were selectively N-acetylated[3] and fractionated on a charcoal–'Celite' column[2] to yield a D-galactose–N-acetylglucosamine mixture (0·80 gm.), disaccharides IV (9 mgm.), V (20 mgm.), trisaccharides I (6 mgm.), II (3 mgm.), III (12 mgm.) and higher oligosaccharides (24 mgm.). Disaccharides and trisaccharides are numbered in order of elution from the charcoal–'Celite' column.

Acid hydrolysis of disaccharide III (1 mgm.) and paper chromatographic analysis of the products showed that III contained D-galactose and D-glucose. Reduction of III with sodium borohydride afforded a disaccharide alcohol the components of which were D-galactose and sorbitol. Hence III was a galactosylglucose. III showed on paper chromatograms R_{glucose} values of 0·33 in butanol/acetic acid/water (40 : 10 : 50), 0·45 in amyl alcohol/pyridine/water (1 : 1 : 1) and on paper ionophoretograms[4] an M_G value of 0·37 in borate buffer pH 10. In each case this behaviour was identical with that of lactose, and III is therefore 4-O-β-D-galactopyranosyl-D-glucose.

The mixture of disaccharides I and II was separated (R_{glucose} values 0·26 and 0·33 respectively) on paper chromatograms irrigated with an amyl alcohol/pyridine/water (1 : 1 : 1) mixture. Acid hydrolysis of II gave D-glucose and D-galactose. Disaccharide II alcohol contained glucose and dulcitol. Since D-galactose must therefore occupy the reducing position in the disaccharide and previous methylation data[1] had established only 3-substituted galactose units, disaccharide II must be 3-O-β-D-glucosyl D-galactose. Its high mobility (M_G 0·70) on borate ionophoresis[4] supports this contention. Disaccharide I contained only D-galactose. Hydrolysis of disaccharide I alcohol gave galactose and dulcitol. Its high mobility (M_G 0·82) on borate ionophoresis[4] supports the assignment of its structure as 3-O-D-galactosyl-D-galactose.

Similar structural investigations established that disaccharides V and IV were identical with 4-O-β-D-glucosyl-2-acetamido 2-deoxy D-glucose and 3-O-β-(2-acetamido 2-deoxy)-D-glucosyl-D-galactose, which had been encountered in previous work[1]. Since the small amounts of disaccharides isolated might have arisen via acid reversion, a control experiment was carried out in which a synthetic mixture of D-galactose, D-glucose, and N-acetyl D-glucosamine (3 : 1 : 2) was submitted to the identical conditions of acid hydrolysis, etc., used in the hydrolysis of the polysaccharide. Only one disaccharide was detected, and this corresponded to disaccharide VI.

Trisaccharide VI (R_{glucose} 0·20 in butanol/acetic acid/water; 0·28 in amyl alcohol/pyridine/water; M_G 0·10 in borate buffer) contained galactose, glucose and N-acetylglucosamine. Partial acid hydrolysis gave as well as these sugars the disaccharides III and V. Sodium borohydride reduction of trisaccharide VI and subsequent partial acid hydrolysis gave galactose, glucose and disaccharide III as the only reducing sugars. Hence trisaccharide VI is probably O-β-D-galactosyl-(1→4)-O-β-D-glucosyl-(1→4) D-(2-acetamido 2-deoxy)-glucose. Trisaccharides IV and V also contained galactose, glucose and N-acetylglucosamine as their component monosaccharides.

Although the fine details of the structure of type XIV polysaccharide must still await the characterization of other trisaccharides and higher oligosaccharides, the following points are significant with respect to the cross-reactivity of anti-type XIV *Pneumococcus* sera and various polysaccharides. Both human blood group A substance[5] and type XIV polysaccharide contain the disaccharide 3-O-β-(2-acetamido 2-deoxy)-D-glucosyl-D-galactose. Anti-type XIV sera do not react with intact A substance, but do so after treatment with acetic acid. Intact human Le^a blood group substance probably cross-reacts with anti-type XIV sera solely because of the presence of non-reducing end groups of D-galactose, since Watkins and Morgan[6] have concluded from inhibition studies that such terminal β-D-galactosyl groups are attached to the 3 position of neighbouring N-acetylglucosamine residues. No evidence has been found for the presence of 4-O-β-D-galactopyranosyl N-acetylglucosamine, which has been found[7] in pig group A substance and is believed by Schiffman, Howe and Kabat[8] to be responsible for cross-reactivity with anti-type XIV sera. The detection by us of 3-O-D-galactosyl D-galactose in type XIV polysaccharide is consistent with the presence of this disaccharide in lung galactan and other polysaccharides containing galactose which cross-react[9–11] with anti-type XIV sera. However, the cross-reaction of guar gum[12] with anti-type XIV sera appears to be due to the non-reducing α-D-galactose terminal units in this polysaccharide.

We are indebted to Prof. M. Heidelberger for his interest in this work and the gift of type XIV polysaccharide. One of us (M. C. K.) is grateful to the Colonial Products Research Council for the award of a research scholarship.

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BIOCHEMISTRY

Role of Tocopherol, Selenium and Anti-oxidants in the Rat

THE view that α-tocopherol functions solely as a lipid anti-oxidant has long been implicit in the conclusions of many workers (Dam[1] has given a useful summary) and has again been most recently elaborated in the work of Zalkin and Tappel[2]. Other workers, however, have postulated a more specific role for tocopherol in animal tissues. We have already shown[3,4] that α-tocopherol and selenium both control ubiquinone-levels in the rat, and wish briefly to report here some work that provides strong evidence for a specific biochemical function of tocopherol, distinct from that of other lipid anti-oxidants.

Groups of six weanling female rats were reared for 10–12 weeks on a vitamin E-deficient diet. Some groups received throughout this period dietary supplements of 0·01 per cent *dl*-α-tocopheryl acetate, or 0·1 p.p.m. of selenium (as selenite) or 0·10 per cent of 'Santoquin' (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline). Other groups were given 80 mgm. of 'Santoquin' or 10 mgm. of α-tocopheryl acetate 48 hr. before all groups were killed. Tissues were analysed for ubiquinone and tocopherol by previously described methods[5]. Table 1 shows that, whereas tocopherol and selenium (which usually increased tissue tocopherol-levels) markedly increased ubiquinone in heart and liver, 'Santoquin' was inactive. Similar effects were found in other tissues.

Table 1. Effect of α-Tocopherol (α-Toc.), Selenium and 'Santoquin' on Ubiquinone (UQ) in Rat Tissues

Group and treatment	Heart UQ (μgm./gm.)	Heart α-Toc. (μgm./gm.)	Liver UQ (μgm./gm.)	Liver α-Toc. (μgm./gm.)
Control	144	1·8	62	0·7
+ 0·01 per cent α-Toc.	170	44·5	92	41·9
+ 10 mgm. α-Toc. dose	165	12·6	83	30·0
+ 0·10 per cent 'Santoquin'	128	1·4	68	6·5
+ 80 mgm. 'Santoquin' dose	151	1·3	65	6·6
+ 0·1 p.p.m. selenium	195	8·2	93	6·8

Table 2 illustrates another experiment. Four groups of 8 female rats were reared on the deficient diet to 4 months of age. One group received a dietary supplement of 0·05 per cent DPPD (N,N′-diphenyl-*p*-phenylenediamine), two other groups dietary 'Santoquin' (at either 0·015 or 0·10 per cent). The animals were killed and livers analysed for ubiquinone and vitamin A. At the same time, *in vitro* peroxide production was measured on three individual livers from each group of the thiobarbituric acid reaction[6], after incubating homogenates for 15 min. at 37°. Optical densities were recorded directly. Both N,N′-diphenyl-*p*-phenylenediamine and 'Santoquin' effectively protected vitamin A (increased liver storage) and both significantly prevented *in vitro* peroxidation in liver. Neither substance significantly increased ubiquinone in heart or liver.

Table 2. The Effect of 'Santoquin' and DPPD on Ubiquinone (UQ) and Vitamin A in the Rat, and their Protective Action on Peroxidation

Group and treatment	Tissue	UQ (μgm./gm.)	Vitamin A (I.U./gm.)	Liver peroxidation* (E at 520 mμ)
Control	heart	208		
	liver	106	116	14
0·05 per cent DPPD	heart	186		
	liver	96	178	8*
0·10 per cent 'Santoquin'	heart	227		
	liver	114	146	11*

*Significantly different from controls ($P = 0·01$).

Dietary selenium did not prevent lipid peroxidation in the rat, whether animals were fed casein-based vitamin E-deficient diets or 'necrogenic' vitamin E/selenium-deficient diets. In a typical experiment, groups of 16 weanling rats were fed a 'necrogenic' bakers' yeast diet, one group being supplemented with 0·05 p.p.m., another with 0·5 p.p.m. of selenium (as sodium selenite), and another with 0·01 per cent *dl*-α-tocopheryl acetate. Three rats in the control group died of liver necrosis at the twenty-third, twenty-sixth and twenty-ninth day; selenium or tocopherol fully protected the other animals. Livers were examined at 20 and 26 days for *in vivo* and *in vitro* peroxidation and the results show (Table 3) that, in contrast to tocopherol, selenium had no significant protective effect on peroxide formation in rat liver.

Table 3. Effect of Selenium on Lipid Peroxidation of Rat Liver

Group and treatment	Liver peroxides (E at 520 mμ) *in vivo*	*in vitro*
Control	6 (4–6)	9 (8–11)*
+ 0·05 p.p.m. selenium	6 (4–8)	10 (8–14)*
+ 0·5 p.p.m. selenium	6 (4–6)	11 (8–11)*
+ 0·01 per cent α-tocopheryl acetate	4, 4, 4	7, 6, 7†

* Mean and range of 7 determinations. † Individual determinations.

These experiments suggest that the mechanism(s) by which α-tocopherol and selenium increase ubiquinone in the rat can be divorced from the general anti-oxidant function of the former substance. Under conditions in which two powerful anti-oxidants have been demonstrated to carry out their function, they were unable to influence ubiquinone; selenium, on the other hand, had no demonstrable anti-oxidant properties, but a tocopherol-like effect on ubiquinone. This work will be reported in full elsewhere.

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Role of Selenium in Relation to Ubiquinone in the Rat

In a preliminary communication[1] and in more detailed work[2], we have shown that α-tocopherol and selenium independently control ubiquinone-levels in the rat, and a similar relation between tocopherol and ubiquinone has been found in the rabbit[3]. We have proposed a theory of vitamin E function which can be briefly summarized as follows. Although animal species vary considerably in their requirements for tocopherol, an even more important variation exists in individual tissue requirements for the vitamin, and in the rates at which tissues become depleted in vitamin E deficiency. Thus, in the rat, uterus normally contains a relatively high concentration of tocopherol and is rapidly depleted, whereas, in the rabbit, skeletal muscle is the tissue most readily depleted. In both these tissues, diminished ubiquinone-levels are the result and may be causally related to the specific syndromes of vitamin E-deficiency, fœtal resorption in the rat and muscular dystrophy in the rabbit. The remarkable species differences in vitamin E deficiency are, according to this hypothesis, a reflexion of local tissue tocopherol deficiencies.

We have now studied the implications of such a hypothesis with respect to the role of selenium. Selenium prevents or cures some symptoms of vitamin

Table 1. EFFECT OF ORALLY ADMINISTERED SELENIUM AND α-TOCOPHEROL (α-TOC.) ON UBIQUINONE (UQ) IN FEMALE RATS

Group and treatment	Heart UQ (μgm./gm.)	Heart α-Toc. (μgm./gm.)	Ovary UQ (μgm./gm.)	Ovary α-Toc. (μgm./gm.)	Uterus UQ (μgm./gm.)	Uterus α-Toc. (μgm./gm.)
Control	30	0·5	48	18	33	0·8
+ 40 μgm. selenium	58	1·1	76	16·8	36	2·4
+ 10 mgm. tocopheryl acetate	132	4·5	88	61·0	50	6·9

Table 2. EFFECT OF DIETARY SELENIUM AND α-TOCOPHEROL ON UBIQUINONE (UQ) IN FEMALE RATS

Group and treatment	Heart UQ (μgm./gm.)	Liver UQ (μgm./gm.)	Kidney UQ (μgm./gm.)	Uterus UQ (μgm./gm.)
Control	144	62	56	13
+ 0·1 p.p.m. selenium	195	98	66	12
+ 0·01 per cent α-tocopheryl acetate	170	92	70	22

E deficiency, its effect varying from species to species. Some syndromes, such as hepatic necrosis in the rat[4] and exudative diathesis in the chick[5], are prevented by either selenium or tocopherol. Other syndromes, such as gestation-resorption in the rat, are only preventable with tocopherol[6]; yet a third group of syndromes, such as muscular degeneration in lambs[7], may well be primarily selenium-deficiencies, only partially responsive to tocopherol. These differences must be explained if an understanding of the role of either compound is to be reached.

In a typical experiment, three groups of 8 female rats (3½ months old) were fed a 'necrogenic' fat-free yeast/sucrose diet designed to reduce tocopherol, selenium and ubiquinone in the animals' tissues to very low amounts. Rats in one group were dosed with 20 μgm. of selenium (as selenite) 48 and 24 hr. before death, and a second group with 5 mgm. of *dl*-α-tocopheryl acetate at the same times. The three groups were killed together. Hearts, uteri and ovaries were analysed by methods already described[8] (Table 1). Tocopherol-levels in heart and uterus were almost too low to measure; but the ovaries contained much tocopherol—being, with adrenals, the last tissue in the body to retain stores of tocopherol[8]. The ubiquinone concentration in deficient heart was very low, but was increased to high levels with α-tocopherol. Selenium had a similar effect (but less marked, due to the smallness of the dose). Selenium and tocopherol both markedly increased ubiquinone in the ovaries, but in uterus, although tocopherol was fully active in increasing ubiquinone, selenium was without effect. In another experiment groups of 6 female rats were reared for 10–12 weeks on a vitamin E-deficient diet. One group received a dietary supplement of 0·01 per cent *dl*-α-tocopheryl acetate, a second a supplement of 0·1 p.p.m. of selenium (as selenite), while a third group acted as controls. Both selenium and tocopherol increased ubiquinone in heart, kidney and liver; only tocopherol had an effect in uterus (Table 2).

While the reason for the failure of selenium to influence ubiquinone in the rat uterus but not in the ovary, which have a common blood supply, is unknown, it is not suggested that this is directly the reason for its failure to prevent fœtal resorption in the rat; nor do we believe that ubiquinone enhancement in uterus by tocopherol explains the latter's action in preventing resorption. Rather, we regard the ubiquinone effect as diagnostic evidence of the ability or otherwise of these two substances to reach the embryos, where ubiquinone synthesis at a certain stage might be extremely critical. This work will be reported in full elsewhere.

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Tocopherol, Ubiquinones and Ubichromenols in Yeasts and Mushrooms

DURING work on vitamin E, we have been interested in obtaining animal diets rigorously freed of tocopherol. Methods of analysis now available[1,2] allow amounts of tocopherol much less than 1 μgm./gm. of diet to be determined and have already shown that many constituents of 'vitamin E-free' diets in fact contain appreciable amounts of tocopherol. Of particular interest is yeast, which has usually been held to be devoid of tocopherol (for example, Forbes and György[3]). When several kinds of commercial dried bakers' yeast were analysed by the above methods they were found to contain small quantities of α-tocopherol (of the order of 0·3 μgm./gm.) Eventually, fresh compressed bakers' yeast (Distillers Co., Ltd., 3 kgm.) was lysed by a method[4] using diethylamine as catalyst, and the solid residue was extracted with hot ethyl acetate. The extract was purified and analysed[1] for α-tocopherol, ubiquinone-30 and ubichromenol-30. In Table 1 the results are compared with those found on a replicate batch of yeast autolysed with ethyl acetate and extracted with hot benzene–alcohol (a method[5] used for ergosterol). It would appear that the former method is superior to the latter. The ubichromenol-30 was identified by comparing it with a synthetic specimen, prepared from ubiquinone-30 by Links's method[6]. Certain batches of yeast appeared to contain ubiquinone-35 instead of ubiquinone-30. The identity of the α-tocopherol was confirmed by isolating it chromatographically. The substance was strongly reducing, ran identically with α-tocopherol in two chromatographic systems, had an ultra-violet maximum at 291 mμ and an infra-red spectrum consistent with it being α-tocopherol.

Since it appeared possible that the tocopherol might have been of exogenous origin, samples of beet and cane molasses (the main organic constituents used for the commercial production of this yeast) were examined. Several hundred grams of each, dissolved

Table 1. TOCOPHEROL, UBIQUINONE AND UBICHROMENOL IN YEAST, ETC.

Material	Ubi-quinone (μgm./gm.)	Ubi-chromenol (μgm./gm.)	α-Toco-pherol (μgm./gm.)
Dried baker's yeast (D.C.L.)	1·1	0·2	0·3
Compressed yeast (D.C.L.)			
(*a*) Diethylamine-treated	2·9	5·2	0·8
(*b*) Ethyl acetate-treated	0·2	0·5	0·2
Cane molasses	Trace	None found	0·2
Compressed bakers' yeast, laboratory grown	32·9	None found	0·8
Dried *Torula* yeast (American, type *B*)	341	420	2·5
Dried *Torula* yeast (American, type 361)*	137	349	4·2
Dried *Torula* yeast (British)	50	65	Trace
P. campestris (cap)	4·7	0·5	1·1
(gills)	7·2	0·4	1·2

* A special yeast produced for high content of vitamin B.
D.C.L., Distillers Co., Ltd.

in water, were extracted in a continuous extractor with ethyl acetate. Analysis of the extracts showed that cane molasses did in fact contain a little α-tocopherol (Table 1), but beet molasses did not. In order to arrive at an unequivocal answer to the problem of the origin of yeast tocopherol, a culture of the commercial yeast was grown in the laboratory on a medium in which the only energy source was pure sucrose. Analysis of the compressed cake (300 gm.), lysed with diethylamine as before, confirmed the presence of α-tocopherol (identified as previously). Considerably more ubiquinone than in the commercial sample was also found, the increase probably being due to the more efficient aeration used in the laboratory, as found by Sugimura and Rudney[7].

Torula yeast (*Torula utilis*) has also been widely used as a constituent of vitamin E-free diets. Two commercial samples of dried *Torula* yeast (Lake States Yeast Corp.) were examined. They both contained considerable α-tocopherol, and were remarkable because of their large ubiquinone and, in particular, ubichromenol contents (Table 1). Yeast is the first natural substance we have so far encountered in which the concentration of ubiquinone is exceeded by that of ubichromenol. The latter is clearly the unidentified tocopherol-like substance previously reported in yeast by Cowlishaw and Prange[8]. Paper chromatographic separation of the *Torula* extracts revealed 3 ubiquinones and 3 ubichromenols. The former consisted mainly of ubiquinones-45 and -35, as already described[9], with a trace of ubiquinone-30. The three ubichromenols were isomeric with the quinones. All six substances were compared chromatographically with authentic samples, followed by individual isolation and determination of their ultraviolet spectra. A sample of British *Torula* yeast was examined (Table 1) and found to contain considerably less of the lipid constituents. However, this yeast also contained more ubichromenol than ubiquinone.

The common mushroom (*P. campestris*) contained amounts of ubiquinone, ubichromenol and α-tocopherol very similar to those in bakers' yeast (Table 1). It seems, therefore that, although in a previous survey[10] it was suggested that the synthesis of α-tocopherol might be limited to chlorophyll-containing organisms, this view can no longer be held.

We wish to thank the Lake States Yeast Corp., Rhinelander, Wisconsin, for their generosity both in providing specimens of *Torula* yeast and their useful information; also Distillers Co., Ltd., for kindly giving us samples of their cane and beet molasses. We also thank S. A. Price and L. Gare of these Laboratories, who provided us with yeast grown on a defined medium.

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An Unidentified Ubichromenol-like Lipid Constituent of Bakers' Yeast

DURING investigations of the unsaponifiable fraction of bakers' yeast (Distillers Co., Ltd.) by two-dimensional chromatography[1], a heavy spot was observed on the papers in a position very close to that expected for ubichromenol-30, which substance is present only in very small amounts in this yeast (preceding communication). The new substance was —like ubichromenol-30—slowly reducing to ferric chloride–dipyridyl; but, on papers pretreated with zinc carbonate containing a trace of sodium fluorescein[2] is weakly fluorescent under ultra-violet light, whereas the ubichromenol is observed as a quenching spot under the same conditions. By a combination of adsorption and reverse-phase partition paper chromatography, a few milligrams of the substance were obtained in a reasonable state of purity. Long runs in two reverse phase paper systems (*a*) liquid paraffin/95 per cent ethanol, and (*b*) olive oil/95 per cent ethanol, clearly distinguished the new substance from ubichromenol-30; the R_F values for these two substances in the latter system were 0·64 and 0·46, respectively. The new substance had absorption maxima at 255 and 282 mμ, with minima at 267 and 315 mμ (in ethanol). In the presence of dilute ethanolic alkali, the 282-mμ peak disappeared and a new peak at 345 mμ appeared. Well-defined isosbestic points occurred at 271 mμ and 322 mμ. These changes are similar to those described[3] for ubichromenol-50, with some displacement of the points, and probably indicate the presence of a phenolic OH grouping as in the latter. The infra-red spectrum had bands at ν 2,899, 2,809, 1,455, 1,373, 1,261, 1,208 and 1,059 cm.$^{-1}$, similar to those found in a specimen of ubichromenol-50 (kindly given by Prof. R. A. Morton). Substance 282 (so-called because the single peak at 282 mμ characteristically distinguishes it from the ubichromenols) was chromatographically distinct from the fluorescent substance, 3-formylindole, which is present in certain animal tissues[4] and has $\lambda_{\max.}$ 282 (in cyclohexane) and $\lambda_{\max.}$ 294 (in ethanol). Substance 282 was found to be strongly active, like α-tocopherol, in the *in vitro* erythrocyte hæmolysis test.

Forbes and György[5] have already isolated from bakers' yeast a blue fluorescent substance, which has some vitamin E-like activity and is also strongly active in the hæmolysis test. This substance has been identified[6] as 2-(6-hydroxy-2-methoxy-3,4-methylenedioxyphenyl)-benzofuran and is clearly distinguishable from substance 282 on chromatographic and spectral evidence. In fact, those are not the only two fluorescent substances in the non-saponifiable fraction of bakers' yeast: yet a third non-reducing substance, strongly fluorescent, is present and has chromatographic properties almost identical with those of α-tocopherol.

It is interesting that substance 282 occurs in bakers' yeast and also in the mushroom (*P. campestris*), both of which contain very little ubichromenol. *Torula* yeast, on the other hand, which contains much ubichromenol (preceding communication), contains no detectable substance 282.

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Electrophoretic Behaviour of Rat Serum Amylase

Determinations of the electrophoretic behaviour of human serum amylase[1] showed that the largest amount of amylase migrated with the albumin fraction and that a significant amount also migrated with the γ-globulins. Our preliminary observations[2] indicated that this was not the case for rat serum. The Delcourts[3] found that this was also not true for mouse serum, where about 75 per cent of the total serum amylase migrated with β_1- and β_2-globulins.

Sera were obtained from both normal rats and those suffering severe liver damage from treatment with N-nitrosodimethylamine[2,4]. The proteins of these sera were separated by paper electrophoresis with a Spinco paper electrophoresis apparatus, Model *R*, using a new filter paper, Schleicher and Schuell 2043*A gl*, thinner than the Whatman paper previously used[1]. The protein fractions were eluted with 0·9 per cent sodium chloride and analysed for amylase by a micro modification[1] of Van Loon's amyloclastic method[5].

As indicated in Table 1, the largest fraction of the serum amylase recovered was that associated with the β-globulin (70 per cent) and a lesser amount (about 25 per cent) with the γ-globulins, a pattern somewhat similar to that found for mouse serum[3]. The leading fractions contained amounts so small as to be almost insignificant. With sera from rats with liver damage, the amylase in the β- and γ-globulin fractions was reduced to less than 30 per cent of that in the normal sera, while the very small amounts in the albumin and α-globulin fractions remained essentially the same as in the normal.

Table 1. Distribution of Amylase in Rat Serum

Serum fraction	Amylase units/100 ml. serum	
	Normal*	N-nitrosodimethylamine* treated
Albumin	24	35
α_1-Globulin	19	19
α_2-Globulin	31	26
β-Globulin	1,012	287
γ-Globulin	337	93
Total of all fractions	1,443	460

* Five animals in each group. Duplicate determinations on each animal.

The fact that liver damage leads to marked reduction in the amylase of the β- and γ-globulin fractions might be interpreted as indicative that the amylase normally found in these fractions is produced in the liver. Because of the uncertainties in the interpretation of measurements of paper electrophoresis caused by protein–protein and protein–paper interactions, we would be cautious about making this interpretation unequivocally from these data alone. However, this interpretation would strengthen our previous conclusion from other data[2,6,7] that the liver is a source of amylase production.

Whatever the conclusions concerning the relationship of the liver to serum amylase, we wish to point out that the distribution pattern of serum amylase may vary from one species to another. That for the rat is definitely different from that for man.

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Identification of 3,4,5-Trimethoxyphenylacetic Acid as the Major Metabolite of Mescaline in the Dog

Mescaline (3,4,5-trimethoxyphenylethylamine), an alkaloid obtained from *Anhalonium lewinii* and *Trichocereus terscheckii*, has been the subject of many investigations. After oral administration of mescaline to dogs, Slotta and Müller[1] were able to isolate 38 per cent of the mescaline as 3,4,5-trimethoxyphenylacetic acid but were unable to detect any of the parent compound in the 24-hr. urine. These results could not be confirmed by Cochin and co-workers[2], who reported that only 'trace' quantities of 3,4,5-trimethoxyphenylacetic acid were excreted in this species, but that 28–46 per cent of a dose, administered parenterally or orally, was excreted as the parent compound. Others[3-6] have reported similar findings of unchanged mescaline in the urine of human beings receiving the drug. The metabolism of this drug was re-investigated in the dog employing 8-^{14}C mescaline.

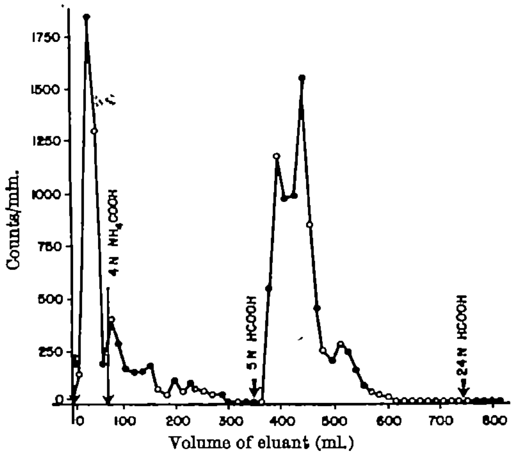

Fig. 1. Fractionation of acidified urine on 'Dowex-1' formate resin

Labelled mescaline (15 µc.) in a dose of 20 mgm./kgm. was administered intraperitoneally to mongrel female dogs anæsthetized with pentobarbitone sodium (35–40 mgm./kgm.). Urine was collected via an indwelling Foley catheter. Aliquots of urine (0·1–1·0 ml.) were combusted and assayed for radioactivity in a 250-ml. ionization chamber (Nuclear-Chicago model 6000). Excretion of radioactivity was determined for periods up to 13 hr. following administration of labelled mescaline. The values for radioactivity excreted during these experiments range from 55 to 62 per cent. Peak urinary excretion of radioactivity occurred within 2 hr. after administration of the drug.

Fractionation of acidified urine (pH 2–3) on 'Dowex-1' formate resin revealed the presence of two major peaks of activity (Fig. 1). The first peak of radioactivity when re-chromatogrammed on Whatman No. 52 paper (solvent system: water/acetic acid/butanol, 4 : 1 : 4) demonstrated an R_F value of 0·71–0·74, whereas mescaline had an R_F of 0·72 when chromatogrammed under similar conditions. The second peak of radioactivity, representing approximately 60 per cent of the excreted radioactivity, was eluted from the resin column with 5 N formic acid and had an R_F value of 0·91–0·92 when examined by paper chromatography.

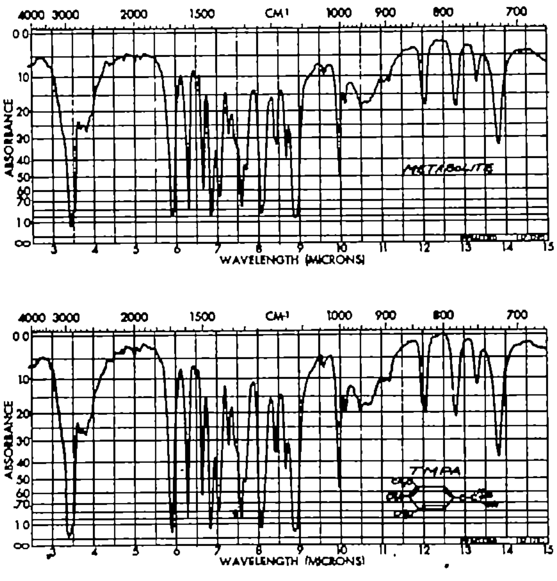

Fig. 2. Infra-red spectra of trimethoxyphenylacetic acid and metabolite of mescaline isolated from the urine of the dog

Acidified, unfractionated urine, or material corresponding to the second peak of resin-fractionated urine, was extracted with chloroform and/or diethyl ether. After evaporation of the latter solvent, the residue was washed with petroleum ether ('Skellysolve B') and then sublimed under vacuum. White crystalline material, melting point 118–119·5° C., was obtained. A mixed melting point with authentic 3,4,5-trimethoxyphenylacetic acid (melting point 118·5° C.) showed no depression. The infra-red absorption spectra of the metabolite and authentic 3,4,5-trimethoxyphenylacetic acid were identical (Fig. 2). Elemental analysis of the metabolite revealed the following values: found: C, 57·51; H, 6·10; N, 0·0; O (by difference), 36·39; calculated: C, 58·40; H, 6·20; N, 0·0; O, 35·40.

These results clearly indicate that deamination with resultant formation of a carboxylic acid is the major pathway of metabolism of mescaline in the dog, but that a significant amount of unchanged drug is also excreted in this species.

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Method of dissolving Triacetyloleandomycin for the Assay of Sensitivity of Micro-organisms

The usual *in vitro* tests of the sensitivity of bacteria to antibiotics are not applicable to triacetyl-oleandomycin (TAO), known in Great Britain as 'Evramycin' and in the United States as 'Cyclamycin', since it is almost insoluble in water. In spite of this, TAO is known to be clinically effective, and after oral administration it attains a high concentration in the blood.

It seems that in order to enter the blood-stream and to reach the peak-concentration in as short a time as about 2 hr., this antibiotic must be converted into an absorbable form very soon after ingestion, either in the stomach or in the upper segment of the intestine.

My experiments *in vitro* showed that TAO is insoluble in the duodenal juice, but it dissolves readily in the gastric secretion, samples of filtered, unfiltered and also of boiled gastric juice being equally effective solvents. This suggests that gastric hydrochloric acid is the effective substance.

TAO dissolves readily in $N/10$ hydrochloric acid (a concentration corresponding to that found in normal gastric juice), but neither gastric juice nor $N/10$ hydrochloric acid could be used as solvents of TAO for assays of sensitivity, since these reagents alone produce narrow zones of inhibition of bacterial growth and may cause considerable alteration and discoloration of complex media.

A series of tests with a wide range of hydrochloric acid concentrations showed that $N/100$ hydrochloric acid had no effect on media or micro-organisms, and dissolved TAO satisfactorily. To provide a margin of safety, however, $N/200$ hydrochloric acid is recommended as the solvent for TAO in sensitivity assays.

Triacetyl-oleandomycin requires an acid medium for its solubilization, and because of this one may speculate whether the speed of absorption and effectiveness of this antibiotic could be increased by dissolving it beforehand or supplementing it with dilute hydrochloric acid during its oral administration, especially in patients with achlorhydria or hypochlorhydria.

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Crystalline α-Kojibiose

Kojibiose (2-O-α-D-glucopyranosyl-D-glycopyranose) was first detected in saké and its moromi[1], and it was isolated from hydrol[2], koji extract[3] and honey[4] as its crystalline α- and β-octaacetates. Chemical synthesis[5],[6] of this sugar was also achieved. But the crystallization of free sugar was hitherto unsuccessful, so its properties such as melting point, specific rotation or its form of crystal were still unknown. We have now succeeded in crystallizing kojibiose and examining its properties.

The same method as reported previously[7] was used for preparation of the kojibiose; but in this work the culture medium of *Leuconostoc mesenteroides* contained sucrose (9 per cent) and lactose (15 per cent) instead of sucrose (2 per cent) and lactose (10 per cent), and then a branched trisaccharide (O-β-D-galactopyranosyl-(1 → 4)-O-[α-D-glucopyranosyl-(1 → 2)]-D-glucopyranose) and lactosucrose[8],[9] (O-β-D-galactopyranosyl-(1 → 4)-O-α-D-glucopyranosyl-(1 → 2)-D-β-fructofranoside) were obtained together. After removal of most of the lactose and dextran by addition of an equal volume of ethanol, the supernatant was concentrated and fractionated by carbon column chromatography. 95 gm. of lactosucrose and 140 gm. of the branched trisaccharide contaminated with a small amount of lactosucrose were obtained from 15 litres of the culture medium. The contaminated lactosucrose in the branched trisaccharide fraction was hydrolysed to lactose and fructose with 0·5 N sulphuric acid at 60° C. for 30 min. and was removed by carbon column chromatography. 97 gm. of the branched trisaccharide was obtained. 10 gm. of the lactase preparation of *Saccharomyces fragilis* (kindly supplied by Dr. Henry Roberts) and 90 gm. of the above branched trisaccharide were incubated in 1 litre of phosphate buffer (pH 6·2), and 48 gm. of amorphous kojibiose was obtained by carbon column chromatography.

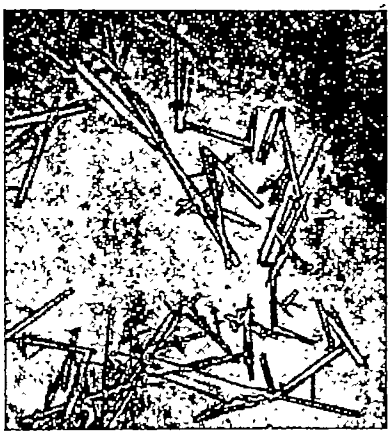

Fig. 1

This crude kojibiose was dissolved in 200 ml. of hot methanol, and the solution was refluxed gently, followed by filtration of the insolubles on 'Celite', and was left to cool at room temperature and then the kojibiose crystallized, 30·5 gm. of crystalline free sugar was obtained. Recrystallization was carried out by dissolving 10 gm. of the above crystals in 15 ml. of hot water and adding 35 ml. of methanol after being decolorized with charcoal. The crystals obtained took the form of fine prisms (Fig. 1). On drying in vacuum at 100° C. for 2 hr. over phosphorus pentoxide, crystalline kojibiose, which was somewhat hygroscopic, showed a melting point of 187–88° C. (uncorrected) and $[\alpha]_D^{18} = +162° \rightarrow 137°$ (17 hr.) (c., 2·1 in water) (calculated for $C_{12}H_{22}O_{11}$: C, 42·11; H, 6·48 per cent; found: C, 42·22; H, 6·48 per cent). The downward mutarotation showed that this crystalline kojibiose was α-isomer. This fact was further confirmed by formation of its acetyl derivative. Namely, only α-kojibiose octaacetate (m.p. 166° C.) was obtained when the acetylation was carried out at a low temperature by using pyridine as catalyst, while both α-kojibiose octaacetate (m.p. 166° C.) and β-kojibiose octaacetate (m.p. 117° C.) were obtained by the usual method using sodium acetate. Neither acetate showed depression of melting point on admixture with the authentic samples synthesized chemically by Matsuda[5].

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PHYSIOLOGY

Plastic Changes of Spinal Monosynaptic Responses from Tenotomized Muscles in Cats

In an attempt to reduce muscle afferent activity, the tendons of some hind-limb muscles of cats were cut. It was postulated that this would prevent the development of effective tension, reduce passive stretching, and so induce relative disuse of the central synaptic connexions of the muscle afferent nerves.

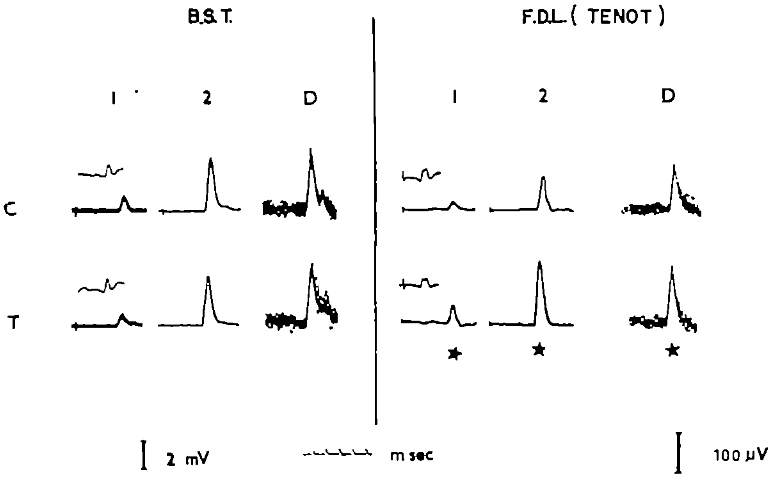

Fig. 1. All reflexes in this figure were taken from one typical experiment. Columns 1 and 2, respectively, are monosynaptic reflex responses for each nerve recorded from *L7* ventral roots before and after tetanic conditioning at 400 c./sec. for 10 sec. The afferent volleys from the cord dorsum are inset. *C*, Control side; *T*, tenotomized side. Biceps-semitendinosus nerve (*B.S.T.*) provided a bilateral control. Flexor digitorum longus (*F.D.L.*) was tenotomized. Dorsal spinocerebellar tract responses occupy columns *D*. Stars indicate enhanced responses from tenotomized muscle

At initial aseptic operations of 23 cats (inset Fig. 2) tenotomy was performed by excising about 10 mm. of the tendons of the following muscles on one side: triceps surae, flexor digitorum longus, and flexor hallucis longus. A dummy operation was made on the contralateral side.

One to four weeks after tenotomy, the spinal monosynaptic reflex responses were tested by two methods during the final acute experiments under pentobarbitone anæsthesia.

(1) The skin of both hind-limbs and the back was formed into pools which were filled with warmed paraffin oil. The cut nerves from the tenotomized and also from the control muscles (biceps-semitendinosus, quadriceps femoris, and peroneal group) in both hind-limbs were stimulated electrically. The reflex responses were recorded monophasically from the central cut ends of ventral roots *S1*, *L7*, *L6*. Further details of methods are the same as those given in a previous communication[1].

(2) Monosynaptic responses in the dorsal and ventral spinocerebellar tracts were also recorded[2]. The dorsal funiculi were dissected off the spinal cord between *T12* and *L2*, and the remaining portion of the cord was then hemisected longitudinally[3]. Dorsal spinocerebellar tract responses to both group 1*a* and 1*b* afferent volleys were recorded from the ipsilateral half-cord[3], while the ventral spinocerebellar tract reflexes from only group 1*b* afferents were recorded in the contralateral half-cord[4].

In Fig. 1 the typical monosynaptic reflexes recorded monophasically from ventral roots show the symmetry of control reflexes evoked by biceps-semitendinosus volleys into both ventral roots By contrast reflexes evoked from the nerve to flexor digitorum longus muscle are, significantly, 50–100 per cent larger from the nerve to the tenotomized muscle (Fig. 1). Though the conduction velocities for the afferent volleys (inset) were equal, a slight shortening of the central latency (mean about 0·1 msec.) was observed in reflex responses evoked from nerves to tenotomized muscles. The results described for biceps-semitendinosus and flexor digitorum longus were duplicated by the other muscles tested. Fig. 2 gives further details. These results are in close agreement with those obtained by Beranék and Hnik[5], who used similar techniques.

Spinocerebellar tract responses evoked by biceps-semitendinosus and flexor digitorum longus volleys in the same experiment (Fig. 1) illustrate the pooled results shown in Fig. 2. There is symmetry of the control dorsal spinocerebellar tract responses from hamstring, peroneal, deep tibial nerves, and of the ipsilateral ascending mass discharges from a skin nerve, sural. The nerves to tenotomized muscles, however, show a 45–55 per cent enhancement of their dorsal spinocerebellar tract responses with no significant change in ventral spinocerebellar tract responses.

Since the dorsal spinocerebellar tract responses arise more from group 1*a* than 1*b* muscle afferent fibres and since ventral spinocerebellar tract has exclusively 1*b*

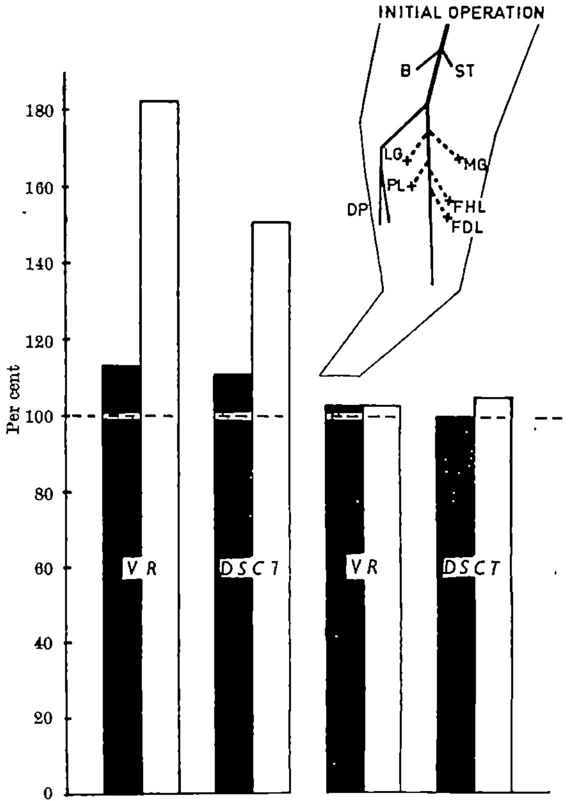

Fig. 2. Inset is a diagram of the initial operative procedure showing the nerves to tenotomized (+ – – –) and normal (——) muscles. Monosynaptic responses recorded from ventral roots *VR* and dorsal spinocerebellar tract response *DSCT* from ipsilateral half-cord. The results depicted in the histogram are averages of pooled reflex responses from 21 animals in the first group and 8 in the second group. The ordinate indicates the mean percentage ratio of corresponding reflexes from tenotomized side/normal side for the groups of nerves to normal (filled columns) and tenotomized (open columns) muscles. Thus 100 per cent indicates bilateral symmetry and a ratio of 1·00. The mean enhancement of *VR* and *DSCT* responses from tenotomized muscle is statistically significant, $P < 0·01$, and is prevented by spinal transection with tenotomy

connexions, our results suggest that the group 1*a* muscle afferents from the muscle spindle are responsible for the enhancement of dorsal spino-cerebellar tract monosynaptic reflex responses, which thus parallels the increased monosynaptic reflexes in ventral roots.

Comparison of muscle wet weight in both hind-limbs of all cats revealed that all muscle groups in the tenotomized leg wasted to some extent. The amount of wasting of tenotomized muscles themselves was much greater and persisted over several weeks, while control muscles on the tenotomized side began to recover their small weight losses after 9–10 days.

Since little is known of proprioceptor function in tenotomized muscles, preliminary attempts were made (in 40 muscles from 10 cats) to analyse the afferent activity from chronically tenotomized muscles by recording directly from the distal segment of cut muscle nerves. A significant 25–40 per cent increase was observed in spontaneous afferent spike discharges from chronically tenotomized muscles.

In further experiments transection of the spinal cord at *T*10–11 level immediately prior to tenotomy prevented, over the same period of survival, the development of both the reflex enhancements (Fig. 2), and the increased afferent discharges induced by tenotomy alone. This effect of cord section, which abolishes supraspinal gamma efferent activation, suggests that an over-active gamma loop mechanism could be responsible for the increased spindle discharges, which could lead to reflex enhancements.

We conclude from available evidence that tenotomy of muscle results in increase of the monosynaptic responses evoked by group 1*a* afferent volleys. There is some evidence suggesting that this effect is brought about by increased discharges of 1*a* impulses from the chronically tenotomized muscles, that is, that it is due to increased synaptic use. The effects are prevented by spinal cord-section (*T*10–11) at the time of tenotomy.

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Role of Biliary Alkaline Phosphatase in the Guinea Pig

ALTHOUGH the part played by intestinal alkaline phosphatase in digestion and absorption is still not clear, it has long been postulated that the high concentration in the duodenum and intestine is concerned with the absorption of glucose[1]. Following adrenalectomy, Verzar *et al.*[2,3] showed that intestinal alkaline phosphatase was reduced and glucose absorption decreased. Administration of adrenal cortical hormones caused regeneration of the phosphatase.

Alkaline phosphatase is known to be secreted in the bile of mammals and it has been suggested that the biliary enzyme plays some part in digestion[4]. The present experiments were designed to see if there were any relation between biliary and intestinal alkaline phosphatase, in particular to see if the high concentration in the intestine were a result of the adsorption of biliary phosphatase by the mucosa. Such a system might be advantageous in replacing enzyme lost either by a mechanical rubbing off or by attack by digestive enzymes.

Duodenal alkaline phosphatase was therefore compared in normal guinea pigs and in guinea pigs in which the common bile duct had been tied and a gall bladder fistula made. In previous reports of guinea pigs with biliary fistulæ, the animals died within 24 hr.[4]. It was found that operated animals drank very little for the first 48 hr., and, as the daily output of bile from a fistula could reach 150–200 ml. (cf. 500 ml. for an adult man), a control of the fluid balance was planned in an attempt to increase survival times. In practice fluid was injected either subcutaneously or intraperitoneally. The fluid injected was Krebs's physiological saline containing vitamins C and K. In the absence of vitamin K, local hæmorrhages occurred.

Male guinea pigs were given vitamin K (0·1 mgm. 'Synkayvit') and vitamin C (10 mgm.) before their operation. The operation was carried out under nembutal and ether anæsthesia and at the beginning of the operation 20-ml. fluid was injected subcutaneously into the axilla. Immediately following the operation, 20-ml. fluid was injected intraperitoneally and a further 20 ml. given subcutaneously 1 hr. later. Thereafter injections of fluid were given at about 3-hr. intervals in an attempt to keep the body-weight constant. The flow of bile was considerably reduced after 48 hr. and it was found practical to maintain operated animals in a healthy condition for at least eight days.

A number of control and experimental animals were killed and duodenal samples rapidly removed. These were freeze-dried and embedded in paraffin wax *in vacuo*. After sectioning they were fixed in 90 per cent alcohol. The alkaline phosphatase of the brush border of the mucosal cells was estimated quantitatively by the method of Barter and Danielli[5]. Phosphatase activity was expressed in standard units of gm. phosphate hydrolysed per gm. of tissue per min. and the results obtained are shown in Table 1.

The results for operated animals show a consistently higher phosphatase activity compared with those from control animals which have not undergone operation. This result is opposite to that which would be expected if duodenal alkaline phosphatase was partly absorbed from the bile. The higher concentration found may indicate a reduced removal of duodenal phosphatase by mechanical or enzymic factors since the food intake of operated animals was lower than in the control animals. Alternatively, adrenal cortical hormones may have increased in the operated animals and caused the increase in duodenal phosphatase.

Table 1

Guinea pig	Period of biliary fistula (days)	Duodenal phosphatase in standard units			
		Proximal duodenum		Distal duodenum	
		Site 1	Site 2	Site 1	Site 2
1 (control)	—	0 050	0 048	0 09	0 064
2 (control)	—	0·063	0 072	0·032	0·062
3 (control)	—	0 064	0·075		
7	5½			0·29	0·52
				0 44	0·46
8	8	0·17	0 46	0 14	0·22
9	7	0 36	0 25	0 22	0·22
		0 49	0·27		
11	2			0·11	0·15
				0 11	0·17

I wish to thank Dr. R. S. Snell for his help with surgical procedures, Mr. C. H. O'Neill for his help in estimating alkaline phosphatase and Mr. A. T Green and Mr. J. Storey for assistance. I am also grateful to Prof. J. F. Danielli, Dr. J. Stubbs and Dr. W. F. Widdas for helpful discussion.

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Alkaline Phosphatase in Cell Cultures of Human Origin

IN an attempt to define some of the changes which cells undergo in adaptation to growth *in vitro*, work has been carried out on the metabolic and enzymatic activities of a variety of established cell cultures, mainly of human origin. For this work, cells were cultured in several media, including Eagle's medium[1] and *M*199 [2] containing 10 or 15 per cent undialysed human serum, or in a nutrient medium described by Marcus *et al.*[3] containing 20 per cent undialysed human serum. Cells were grown for 6–8 days on glass surfaces at 37° C. in an atmosphere of 5 per cent carbon dioxide in air, and dispersed with 0·05 per cent trypsin in saline. They were collected by centrifugation at 500*g*, washed and resuspended in 0·25 *M* sucrose, and disrupted with a 10-kc./s. Raytheon sonic oscillator for 1–5 min. Cell protein was measured by the procedure of Lowry *et al.*[4].

The initial findings confirmed observations made by Lieberman and Ove[5] with regard to the similarity in specific activities of catalase, glucose-6-phosphate dehydrogenase, rhodanese and β-glucosidase among cell-free preparations of various cell lines. On the other hand, a striking dissimilarity was noted in alkaline phosphomonoesterase activity as measured by the hydrolysis of *p*-nitrophenylphosphate in 1 *M* 2-amino-2-methyl-1-propanol buffer, at *p*H 10·6, according to Lowry's technique[6].

Table 1 summarizes the specific activities for alkaline phosphatase in sonicates of a variety of established human cell lines, including the parental and three clonal strains of the *Z* liver line derived from an infant with congenital atresia of the bile ducts, and of a recently explanted bovine embryonic kidney cell line. As can be noted, alkaline phosphatase activity with sonicates of the Chang liver line[7] is two-fold greater than that of human intestine[8], three-fold greater than that of *KB* (Eagle)[9] and anywhere from 25- to 200-fold greater than that of other cell lines. After one or two successive single cell platings according to the technique of Puck *et al.*[10] several clonal strains of Chang liver have been obtained which show differences in alkaline phosphatase activity. Sonicates of one of these clonal strains, which can be defined by the gross morphological characteristic of a smaller cell size, have a specific activity which is only 1/40 that of the parental strain.

Table 1. ALKALINE PHOSPHATASE ACTIVITY OF MAMMALIAN CELLS IN CULTURE

Cell line	Activity, *p*H 10·6
Chang liver	21·88*
Intestine (Henle)	9·65
KB (Eagle)	6·56
Z Liver-*P.S.*	0·53
Z Liver-1	0·15
Z Liver-2*A*	0·14
Z Liver-4*A*	0·84
HeLa-*S*3	0·12
HeLa-3218*A*	0·22
MAF (Gray)	0·71
Bovine kidney	0·19

* Specific activity = μ*M* *p*-nitrophenyl phosphate hydrolysed per mgm. protein in 30 min. at 38° C. in 1 *M* 2-amino-2-methyl-1-propanol – hydrochloric acid buffer, *p*H 10·6.

Separation of cell sonicates by differential centrifugation in 0·25 *M* sucrose[11] reveals the major portion of activity for all cell lines in the microsomal fraction. Extraction of a 10 per cent microsomal preparation with *n*-butanol[12] yields up to 9–10-fold purification of the phosphatase activity. Kinetic studies with the enzyme from the Chang liver microsomal fraction show the reaction to be zero order with the activity linearly dependent on enzyme concentration over a 100-fold range. The *p*H optimum is 10·6 with a decline to 10 per cent of maximal activity at *p*H 8·0. No activation with Mg^{2+} is found using *p*-nitrophenylphosphate as substrate, but inhibition is observed with Be^{2+}, Zn^{2+}, Mn^{2+}, CN^- and cysteine. Concentrations of 10^{-2} *M* inorganic phosphate are required for inhibition of activity. The enzyme exhibits a broad substrate specificity showing activity against *p*-nitrophenylphosphate, β-naphthylphosphate, β-glycerophosphate, adenosine monophosphate, glucose-6-phosphate and ethanolamine phosphate in decreasing order. Work is in progress to characterize further the physical-chemical properties of these enzyme preparations, some preliminary results of which indicate qualitative as well as quantitative differences in properties of enzymes derived from different cell lines.

During the course of this work, it was noted that the specific activity of alkaline phosphatase in Chang liver sonicates shows some variation over a four-fold

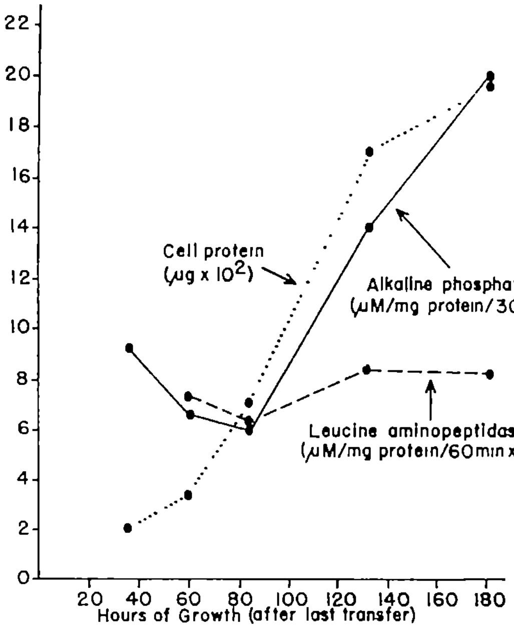

Fig. 1. Change in specific activity of alkaline phosphatase and leucine amino-peptidase in Chang liver sonicates with growth

range, although it is consistently higher than the activity of other cell lines. This variability can be correlated with the duration of growth following their last transfer, and in general, the longer the growth period the higher is the specific activity observed. These findings are illustrated in Fig. 1, where during a single growth-cycle of 180 hr., when cell protein increases ten-fold, the specific activity of leucine aminopeptidase remains constant but the specific activity of alkaline phosphatase rises to a value of 20·00 at 180 hr. of growth after falling from 9·50 initially to 6·00 at 84 hr.

Of interest are the recent observations of induced synthesis of alkaline phosphatase in *E. coli*[13,14], *B. subtilis*[15] and yeast[16] in response to removal of the repressor effect of inorganic phosphate in the growth medium. Preliminary work on inorganic phosphate concentrations in the nutrient medium reveal no alterations during the growth of Chang liver cells which can be correlated with the change of alkaline phosphatase activity. Work is now in progress to define more clearly whether an inducer or repressor mechanism plays some part in the differential activation or synthesis of alkaline phosphatase in these cells and whether such alterations, like those demonstrated by De Mars for glutamine synthetase[17], are characteristic of other enzyme systems.

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Prevention by Vagotomy of Erythrocytosis induced by Hypoxæmia

WHILE investigating the effect of fluid aspiration into the lungs[1], observations were made on erythrocytosis induced by hypoxæmia, which to our knowledge have not yet been described.

Sheep were anæsthetized with 'Thiopentone', intubated, and given 1–3 ml./kgm. fresh water into the trachea. Before and 5 min. following the latter procedure, blood samples were taken from the femoral artery through an indwelling cannula. Arterial oxygen saturation, hæmoglobin and *p*H were determined and arterial oxygen tensions calculated. Observations carried out on 24 intact and 24 vagotomized sheep are reported here. Vagotomy was performed bilaterally on the neck prior to the control period.

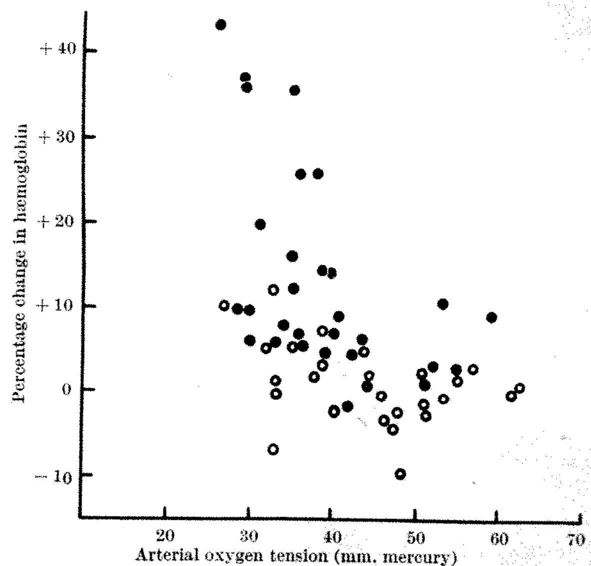

Fig. 1. Relationship of arterial oxygen tension and percentage change in hæmoglobin content in intact (●) and vagotomized (○) sheep 5 min. following the induction of hypoxæmia. Each dot represents one animal. Oxygen tension is given in mm. mercury

Results are shown on Fig. 1. The arterial oxygen tension 5 min. following the inhalation of water was related to the percentage change in hæmoglobin.

Water inhaled into the lungs is rapidly absorbed and remains unaffected by vagotomy[2]. Both groups were given strictly equal quantities of water; the resulting hæmodilution is therefore comparable.

Erythrocytosis appears to commence rather abruptly at an arterial oxygen tension of about 35–40 mm. mercury. This is in good agreement with earlier observations, suggesting a critical oxygen tension of 40 per cent.

In experiments reported in the literature, hypoxæmia was caused by the inhalation of gas mixtures low in oxygen. Alveolar oxygen tension in our animals was invariably normal[1]. It seems justifiable to conclude that arterial unsaturation is in itself a sufficient stimulus to mobilize the stored red cells in the body.

A comparable degree of hypoxæmia failed to cause erythrocytosis in our vagotomized sheep ($r = -0·153$; $P = 0·40$). The nerves were divided well below the carotid sinus: its denervation could scarcely have been responsible for the absence of the reaction.

Splenic[3] and hepatic contraction, and an increase in splanchnic blood-flow, appear to be the accepted mechanism of hypoxic erythrocytosis. Splanchnic stimulation is followed by similar changes, while electric stimulation of the peripheral end of the divided vagus was without an effect. The profound fall in hæmatocrit during anæsthesia[4] and following the administration of chlorpromazine and dihydroergotamine[5] is accompanied by an increase in the red-blood cell content of the spleen.

Barcroft and Stephens[3] suggested that splenic contraction is the effect of hypoxæmia on the central nervous system. Our observations would therefore suggest that it is the absence of an afferent impulse which accounts for failure to observe hypoxic erythrocytosis after vagotomy.

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Relationship of the Sarcoplasmic Reticulum to Sarcolemma in Sheep Cardiac Muscle

SINCE Huxley and Taylor[1] demonstrated that in striated muscle each myofibril segment probably receives an individual stimulus to contract, electron microscope studies have suggested that the most likely pathway for such stimuli from the sarcolemma to the myofibrils is the sarcoplasmic reticulum[2], particularly[3] its transverse tubular elements (also named the 'transverse sarcotubular system'[4]). Theories about this have recently been elaborated and the evidence has been reviewed[5,6]. Such theories are necessarily based on the possibility that the membrane of the tubules is polarized and is capable of undergoing depolarization in the same way as the plasma membrane (inner layer of sarcolemma).

The question of continuity between plasma membrane and sarcoplasmic reticulum is a somewhat vexed one. Such continuity appears to occur in certain non-muscular cells[7,8], and it has been suggested or demonstrated also for both cardiac[2,9–11] and skeletal muscle[12,13]. This could not, however, be proved by serial sections in a major study of skeletal muscle[4], and the theories put forward[5,6] concerning impulse conduction by the sarcoplasmic reticulum are not based on a concept of communication between the lumen of the sarcoplasmic tubules and the extracellular fluid, although Huxley[14] favours such a concept on physiological grounds. Lindner[11] found that the transverse tubules in the dog myocardial cells had triple-layered membranes, like the sarcolemma, and he suggested that these tubules might be continuous with invaginations of the sarcolemma.

We have studied the fine structure of sheep cardiac muscle and we have found evidence of two types of tubular structure within the cell. One type is rather sparse in our material, possibly due to fixation artefact, and consists of delicate irregular small tubules, some of which are in contact (if not actual continuity) with the inner layer of the triple-layered sarcolemma (Fig. 1), in agreement with previously published work[5,9]. The other type is usually seen as cross-sections of tubules, lying between the myofibrils, usually opposite *Z* bands (Fig. 2). These tubules are comparatively large with triple-layered walls, identical in thickness and appearance to the sarcolemma. When the plane of section passes longitudinally between two myofibrils, the transverse tubules are cut longitudinally (Fig. 3), and the walls of some of the more superficial ones can be seen to be continuous with invaginations of the sarcolemma, as suggested by Linder[11]. We feel that the electron micrographs cannot be satisfactorily interpreted in any other way; complete proof of the continuity of the whole transverse tubular system and the sarcolemma will depend on a three-dimensional picture built up from serial sections, but the present findings go far towards confirming Huxley's theories[3,14] of muscle activation.

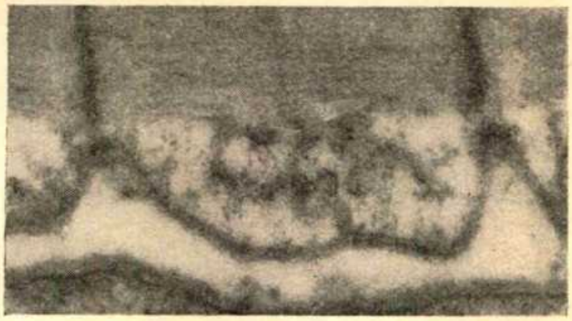

Fig. 1. Electron microscope (Siemens Elmiskop 1) photograph of edge of sheep myocardial cell. Part of a network of fine tubules can be seen, apparently continuous with the plasma membrane (inner layer of sarcolemma). (× 43,000)

This concept makes it much easier to accept the theory of a polarization of the sarcoplasmic reticulum, as it implies that this part of the sarcoplasmic reticulum is actually an integral part of the sarcolemma and necessarily undergoes the same electrical and chemical changes as the sarcolemma. The concept also implies that the lumen of this system of tubules is in communication with the extracellular space, and that the total area of sarcolemma (as Siekevitz[15] has suggested for other cells) is much greater than simply the external surface area of the cell. This may help to explain the great rapidity of the ionic fluxes which take place across the sarcolemma.

One may speculate further whether the alternate contraction and relaxation of the cell provide a pumping of extracellular fluid out of, and into, the system of tubules, and whether this might be of importance in the nutrition of the cell as well as in the electrical events of depolarization and repolarization.

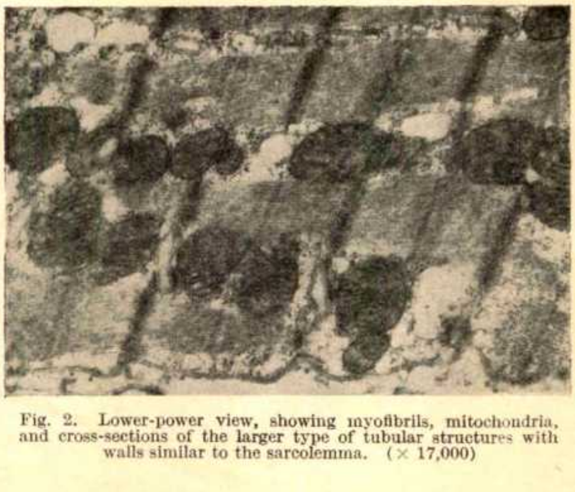

Fig. 2. Lower-power view, showing myofibrils, mitochondria, and cross-sections of the larger type of tubular structures with walls similar to the sarcolemma. (× 17,000)

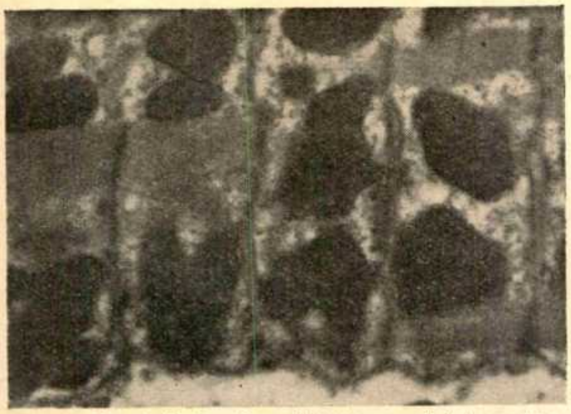

Fig. 3. Longitudinal section which has passed between two subsarcolemmal myofibrils. The walls of one of the longitudinally sectioned tubular elements are continuous with a sarcolemmal invagination, and the lumen communicates with the exterior. (× 12,000)

It is evident that this concept of myocardial cell structure will require a re-appraisal of the physiology of the myocardium and may help to solve some of the problems associated with the electro-chemical phenomena of the heart. It must be emphasized, however, that the present report is based on observations of cardiac muscle from the sheep only, and that the findings do not necessarily apply to voluntary muscle.

One of us (F. O. S.) is indebted to Dr. T. E. Lowe, director of the Baker Medical Research Institute, for suggesting this work, and to Dr. P. L. Bazeley, director of the Commonwealth Serum Laboratories, for access to electron microscope facilities.

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Partition of Calcium and Phosphorus Excretion in the Laying Hen

THE need for surgery as a prerequisite to the separate collection of urine and fæces in birds has restricted our knowledge of their excretion pattern. Recently, the nitrogenous excretions of chickens have been investigated[1], but no data have as yet been published on the partition of calcium and phosphorus excretions. In order to study these excretions in laying hens, colostomies were performed by exteriorizing the distal end of the colon at a point between the ischium and the abdominal midline at the right side, approximately 1 in. anterior to the ventral lip of the cloaca, essentially as described by Ariyoshi[2]. In most birds the operation did not disturb egg production. For the collection of fæces, a polyethylene collar provided with a thread was sutured to the skin surrounding the exteriorized intestine, and a wide-mouth bottle of the same material screwed into the collar.

The hens received the following diet (quantities as percentages):

Table 1

Glucose	59·65	Choline chloride	0·1
Soybean oil meal	20	Vitamin mixtures (ref. 3)	0·25
Dried egg albumen	8	K_2HPO_4	1·1
Refined corn oil	3	$NaH_2PO_4.2H_2O$	1·1
Sodium chloride	0·3	$CaCO_3$	6·5

The assayed calcium and phosphorus contents of the diet were 2·64 and 0·50 per cent, respectively. The feed was supplied *ad libitum* from feeders specially constructed to minimize spillage. Water was supplied by a low-pressure drip system mounted overhead at the rear end of the cage, the 'founts' being activated by contact with the chicken's beak; this system minimizes feed wastage in water[4]. Daily measurements of calcium and phosphorus intake and excretion were done on four surgically prepared hens receiving this diet for seven consecutive days. Calcium was determined by a method using ethylenediamine tetraacetate as described elsewhere[5]. For the estimation of phosphorus the excreta were wet-ashed with 10 *N* sulphuric acid and 30 per cent hydrogen peroxide and phosphorus determined by Gomori's method[6].

The results are summarized in Table 2. The absolute amount of calcium appearing in the urine is similar to the amount of phosphorus, but as a percentage of the ingested mineral, 51 per cent of the ingested phosphorus appeared in the urine, whereas the corresponding average value for calcium was only 7 per cent. More than 58 per cent of the ingested calcium was deposited in the egg (most of it in the shell) and nearly all the calcium retained could be accounted for by the calcium of the egg. As a percentage of excreted mineral, 19 per cent of the excreted calcium and 62 per cent of the excreted phosphorus were found in the urine.

Table 2. CALCIUM AND PHOSPHORUS BALANCE IN COLOSTOMIZED HENS

	Calcium	Phosphorus
	(gm./hen/day)	
Consumption	3·00 ± 0·66*	0·57 ± 0·12
Excretion in fæces	0·95 ± 0·21	0·18 ± 0·03
Absorption†	2·05 ± 0·58	0·39 ± 0·12
Excretion in urine	0·22 ± 0·06	0·29 ± 0·02
Retention‡	1·83 ± 0·56	0·10 ± 0·10
Eggs§	1·75 ± 0·17	0·08 ± 0·00
Balance	0·08 ± 0·15	0·02 ± 0·02
	(Per cent)	
Absorption†	67·8 ± 9·9	67·0 ± 8·1
Retention‡	60·4 ± 10·7	15·5 ± 14·6

* Arithmetic mean ± standard deviation, calculated from individual values (4 hens for 7 days).

† Apparent absorption (consumption minus fæcal excretion) disregarding possible re-excretion of absorbed calcium and phosphorus into the gastro-intestinal tract.

‡ Consumption minus excretion in urine and fæces.

§ Each hen laid six eggs during the seven-day experimental period. The figures for eggs (and balance) denote the total values of the period divided by seven; the standard deviations are based on the averages for each hen thus obtained.

The average apparent absorption was similar for both calcium and phosphorus. It should be kept in mind, however, that the true absorption is probably higher, since the possibility of re-excretion of absorbed minerals into the intestinal tract must be considered. On the other hand, the retention and percentage retention of phosphorus were much lower than that of calcium, and showed a relatively higher variability. This higher variability of phosphorus retention may be connected with the need of the individual bird to draw upon bone material. Since the ratio calcium/phosphorus in bone (2·5 : 1) differs greatly from that required for egg formation (20 : 1), hens having a negative calcium balance, indicating the use of bone calcium for egg formation, are likely to have an excess of phosphorus at their disposal; this will lead to an increased urinary excretion of phosphorus and consequently a low retention of phosphorus, as calculated on the balance sheet. In fact, the only hen on a negative calcium balance (– 0·08 gm.) in this experiment excreted as much as 0·35 gm. phosphorus/day and retained only 0·03 gm.

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RADIOBIOLOGY

Protective Action of Calcium Gluconate against After-effects of X-irradiation on Conidia of *Neurospora crassa*

AN increase in the survival of conidia of *Neurospora crassa* irradiated with X-rays has been found when calcium gluconate is added to the plating medium.

A colonial, microconidial, fluffy strain of *N. crassa* (*UA*–51) was grown on a minimal Vogel medium[1] containing 1 mgm. of calcium chloride per 10 ml. Conidia were harvested after incubation for seven days at 30° C. and suspended in distilled water with a final density of 5×10^6 cells per ml. Seven (10 ml.) samples were exposed to X-ray doses of 240, 720, 1,200, 2,400, 7,200 and 9,600 r. respectively (dose-rate 4 r./sec. at a distance of 25 cm. from the target of a Philips X-ray machine operating at 300 kV. constant potential, with a half-value thickness for the unfiltered beam of 5 mm. aluminium). The conidia from both the irradiated and control samples were plated on minimal medium with and without added calcium gluconate (15 mgm./10 ml.). The amount of light received by the conidia during plating was kept to a minimum and constant for all samples. Six replicate experiments were carried out at each dose. After plating, the conidia were incubated at 30° C. in darkness and germination counts were made on the fifth day.

The addition of calcium gluconate caused an increase in the germination of irradiated conidia when compared with that on the unsupplemented medium (Table 1). This increase in survival was observed at all dose-levels studied and was statistically significant ($P < 0·05$) in seven out of eight doses. The presence of calcium gluconate after irradiation, therefore, lessened the death-rate of spores. Unirradiated conidia showed no significant difference in germination on the two media.

Table 1. EFFECT OF CALCIUM GLUCONATE ON THE PERCENTAGE SURVIVAL OF CONIDIA OF *Neurospora crassa* IRRADIATED AT DIFFERENT DOSE-LEVELS

Dose-level (r.)	Germination of irradiated sample on minimal medium*	Germination of irradiated sample on minimal medium + calcium gluconate*	
0	100	98	
50	132†	196†	$P < 0·001$
240	76	151†	$P < 0·001$
720	46	96	$P < 0·001$
1,200	93†	98	$P < 0·8$
2,400	59	92	$P < 0·001$
4,800	34	79	$P < 0·001$
7,200	44	78	$P < 0·001$
9,600	40	76	$P < 0·001$

* Normalized to germination of unirradiated control conidia as 100. The irradiation experiments with different doses of X-rays consisted of six replicates, each replicate representing 40 conidial platings with a colony count of approximately 100 colonies per plate at zero *r*. The plating efficiencies of all 96 replicates were determined before irradiation, but these results have not been incorporated in this table, no significant differences ($P < 0·01$) having been observed.

† These figures were consistently high in all six replicates.

The plating efficiency of the unirradiated conidial cells is about 35 per cent. The germination of treated conidia has been expressed as a percentage of the germination of unirradiated control conidia. Thus, an increase in plating efficiency as a result of the treatments may appear as a germination percentage of greater than 100.

In addition to the protective action of calcium against the consequences of radiation injury, there was also evidence at certain doses of stimulation of germination by the radiation. In some cases this was seen as an increased efficiency of plating when compared with that at zero dose; in others it was apparent as a reduction in net death of conidia compared with that observed following lower doses (unpublished work). Thus, when conidia were plated on minimal medium without calcium gluconate, the survival percentage following a 50-r. exposure exceeded 100, and survival following a 1,200-r. exposure (while less than that at zero dose) was twice as great as at 720 r. The survival steadily decreased at doses higher than 7,200 r. (zero survival out of 10^6 conidia being obtained with 115,000 r.). Superimposed on the destructive effects of the irradiation there is apparently stimulation induced by radiation of conidia which would not otherwise germinate.

Rixon and Whitfield[2], in work involving increased survival of X-irradiated rats when treated with parathyroid extract, stated that protection against radiation death might be due to the ability of the hormone to increase serum calcium; they (Whitfield and Rixon, personal communication) believe that an increased calcium-level protects the irradiated cell by reducing leakage of cell components through the cell membrane.

The radiation experiments with conidia of *N. crassa* confirm the hypothesis that calcium is a potential protector against death arising from the effects of radiation, and the experiments reported below show that the protection is associated with the prevention of a radiation-induced increase in cell permeability.

Conidia were raised on a medium containing 1·5 mc. of phosphorus-32 per agar slant for 7 days.

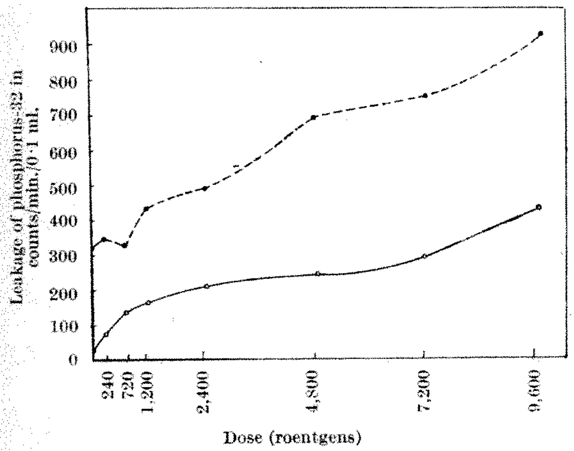

Fig. 1. Protective action of calcium gluconate against leakage of phosphorus-32 from conidia of *Neurospora crassa* after X-ray irradiation. ----, untreated; ———, treated with calcium gluconate

By the end of this time, there was 10^{-3} µµc. phosphorus-32/conidium. When these conidia were irradiated, there was a rapid release of phosphorus-32 into the medium (distilled water) in which the conidia were suspended. The magnitude of this leakage varied with the dose (Fig. 1).

When calcium gluconate was added to the medium on which the conidia were raised (25 mgm./10 ml. medium) (Fig. 1), and also when it was added to the distilled water in which the conidia were irradiated, the post-radiation leakage of phosphorus-32 could be largely prevented even when X-ray doses as high as 7,200 r. were used.

A detailed report of these experiments will be published later. The work was carried out in part during the tenure of a visiting professorship at the Biology Branch of Atomic Energy of Canada, Ltd., Chalk River (Ontario), and was supported in part by a research grant from the National Cancer Institute of Canada. My thanks are due to Dr. J. F. Whitfield and Dr. R. H. Rixon for their interest during these investigations.

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Protective Effects of Di*iso*propylfluorophosphonate against the Lethal Effects of X-rays before or after Irradiation

Pre-treatment with di*iso*propylfluorophosphonate (DFP) suppresses the increase in permeability of the intestinal vessels of rats after abdominal irradiation[1,2] and similar vascular changes due to other types of injury[3]. DFP also reduces gastrointestinal symptoms (diarrhœa), which occur after exposure to X-rays. Since death from gastrointestinal injury is one of the well-recognized forms of acute radiation death, it was decided to test the effects of DFP on rats exposed to whole-body irradiation. Twenty rats were given 975 r. whole-body X-rays with a Philips X-ray apparatus (200 kV. 15 m.amp. 0·5 mm. copper, and 1·0 mm. aluminium filters, *F.S.D.* 30 cm. distance); ten of these animals received a prior injection of DFP before irradiation (3·0 mgm./kgm.).

The results of this experiment (Fig. 1) show that in the first three days after exposure to X-rays 60 per cent of the control, but only 20 per cent of the pre-treated animals had died. By 28 days, however, the same proportion of control and pre-treated animals survived. The early deaths (up to three days) were due to gastrointestinal injuries which manifested themselves in severe diarrhœa and its consequences, while the deaths in the second phase (six days and later) could be attributed to the lethal injuries to the hæmatopoietic system, thus confirming the well-established sequence of radiation deaths[4,5].

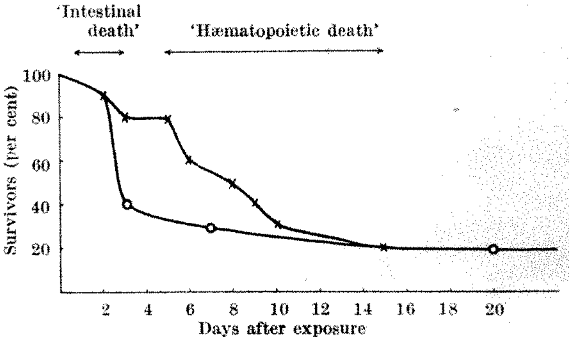

Fig. 1. Effect of DFP on whole-body irradiation. ×, treated with DFP; ○, X-rays only

Thus it appears that pre-treatment with DFP protected the rats against the 'intestinal death' but not the 'hæmatopoietic death'. To test this hypothesis further, rats were irradiated with various doses of X-rays over the entire abdomen, the rest of the animal being shielded so that most of the hæmatopoietic system was protected.

Table 1 indicates that treatment with DFP not only delays but also prevents death in a high percentage of rats exposed to doses of 1,050–1,334 r. of half-body irradiation. Of 60 rats receiving such doses of X-rays 95 ± 2·8 per cent died within four weeks, while of those treated with DFP only 65 ± 6·2 per cent succumbed (difference 30 ± 6·8). DFP proved ineffective when the dose of X-rays was increased to 2,200 r. The protective effect of DFP was the same whether administered before, immediately after exposure or 24 hr. after irradiation (Table 1).

Table 1. Effect of DFP Treatment on the Survival of Irradiated Rats (Abdominal Fields)

Dose (r.)	No. of rats	DFP treatment	Time to reach 50 per cent deaths (days)	Per cent deaths at 7 days	Per cent deaths at end of experiment (28 days)
1,050	12 ♂	0	5·5	58·0	82·5
1,050	12 ♀	0	4·0	75·0	100·0
1,050	12 ♂	Before X-rays	11·5	25·0	58·0
1,050	12 ♀	Before X-rays	11·0	25·0	58·0
1,050	12 ♂	0	5·0	75·0	92·0
1,050	12 ♂	Immediately after X-rays	11·0	33·0	58·0
1,334	12 ♂	0	3·0	92·0	100·0
1,334	12 ♂	Before X-rays	4·5	66·0	75·0
1,334	12 ♂	0	3·0	92·0	100·0
1,334	12 ♂	24 hr. after X-rays	5·5	50·0	75·0
2,200	12 ♂	0	3·5	100·0	100·0
2,200	12 ♂	Before X-rays	4·0	92·0	92·0

These experiments support the theory that DFP selectively protects rats from 'acute intestinal death' even if given *after* exposure to irradiation.

I am grateful to Dr. H. B. Fell and Dr. A. Glucksmann for their advice and encouragement. This work was done at the Strangeways Research Laboratory, Cambridge, during the tenure of a Wellcome Research Scholarship.

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Differential Radiosensitivity in Rice (*Oryza sativa* Linn.) at the Genotypic Level

INFORMATION on differential radiosensitivity of genotypes of a gene locus is scanty[1]. During a course of investigations on the effects of pile radiation on rice, advantage was taken of an anthocyanin pigmented mutant appearing as a heterozygote for albinism in N_1 population of a variety *GEB*-24 to study the relative radiosensitivity of the genotypes at this locus. N_2 seedlings of the mutant segregated into greens and albinos in a 3 : 1 ratio. N_3 plants, homozygous dominant and heterozygous for chlorophyll character, segregated in 1 : 2 ratio, showing that the genes for albinism behaved as simple recessive to normal green. In the first of the two experiments, dry seeds from the heterozygous N_1 plant were irradiated with 5–40 kr. of X-rays from a therapy type X-ray machine operating at 250 kV., 30 m.amp., with 2·0 mm. copper. The seeds, stabilized for moisture content, were placed at a distance of 22·0 cm. from the target and at that distance the dose-rate was 309 r. per min. In the second experiment, in addition to those from the N_1 plant referred to above, seeds of one of its N_2 progeny, known to be homozygous dominant for green, were also irradiated. The dosage-range was 10–50 kr., and the dose-rate at a distance of 13·5 cm. from the target was 650 r. per min.

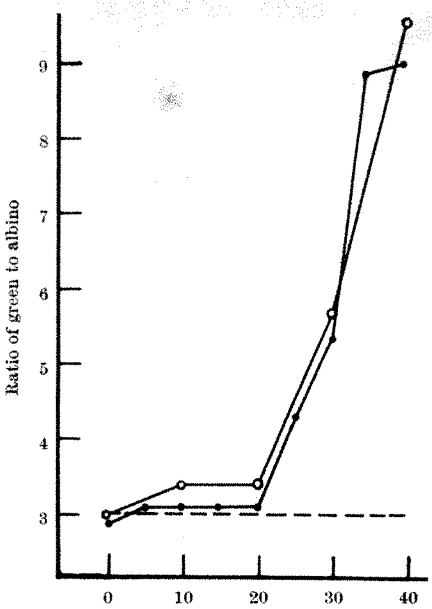

Fig. 1. Ratio of green to albino : – – – –, expected ; ●—●—●, observed in exp. 1 ; ○—○—○, observed in exp. 2

It can be seen from Table 1 that X-ray doses higher than 20 kr. had increasing effects on the rate of reduction of number of seedlings in both the experiments. It was further evident that with high doses the normal ratio, namely, 3 : 1 of green to albino seedlings, was also affected. As the dose increased, the rate of decrease of albinos in relation to greens became significantly higher, giving eventually 9 : 1 and 9·5 : 1 ratios for greens to albinos at 40 kr. in experiments 1 and 2 respectively (Fig. 1). It was evident that this was due to the greater lethality in the albinos, and the seeds carrying the recessive condition were more radiosensitive than the other two genotypes. It is of further interest to note that a difference existed between the homo- and heterozygotes for green in their degree of radiosensitivity. At the dose-level of 40 kr., there was more than four-fold decrease in emergence of seedlings in the mixed population of homo- and heterozygous greens as compared to the homozygous dominant. Presumably the greater radiosensitivity of the recessive gene is expressed even when it is in a heterozygous condition. Whether higher radiosensitivity in the recessive genotype was due to a pleiotropic effect or mutation of a closely linked gene

Table 1. EFFECT OF X-RAYS ON EMERGENCE OF RICE SEEDLINGS
Material, *O. sativa*, var. *GEB*-24. Total number of seeds irradiated per treatment: Exp. 1, 400 ; Exp. 2, 200

Treatments	Exp. 1 No. of seedlings from the N_1 plant 14 days after sowing				Exp. 2 No. of seedlings from the N_1 plant 21 days after sowing				No. of seedlings from an N_2 plant 21 days after sowing
	Percentage emergence of: homo- and hetero-zygous greens	albinos	χ^2	P*	Percentage emergence of: homo- and hetero-zygous greens	albinos	χ^2	P*	Percentage emergence of homozygous green
Control	73·7	24·7	0·003	0·99	69·0	23·0	0	0·99	99·0
5 kr.	64·0	20·5	0·098	0·90–0·80	—	—	—	—	—
10 kr.	70·2	22·7	0·057	0·90–0·80	65·5	19·5	0·384	0·70–0·50	97·5
15 kr.	71·7	23·0	0·106	0·80–0·70	—	—	—	—	—
20 kr.	67·7	21·2	0·239	0·70–0·50	62·5	18·5	0·403	0·70–0·50	97·0
25 kr.	64·0	14·0	8·274	0·01–0·001	—	—	—	—	—
30 kr.	61·0	11·5	14·405	$<$ 0·001	46·0	8·0	5·975	0·02–0·01	85·0
35 kr.	42·5	4·7	22·520	$<$ 0·001	—	—	—	—	—
40 kr.	27·0	3·0	14·400	$<$ 0·001	9·5	1·0	2·682	0·20–0·10	63·0
50 kr.	—	—	—	—	0	0	—	—	0

* Probability of obtaining a ratio different from 3 : 1.

or albinism *per se* could not be ascertained from the present observations.

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Metabolism of Carboxyl-^{14}C-labelled 2,4-Dichlorophenoxyacetic Acid by Bean Stems: Heterogeneity of the Ethanol-soluble, Ether-insoluble Products

THERE is a considerable disagreement in the literature concerning the nature and number of radioactive metabolites which can be isolated from bean plants following exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) labelled with carbon-14 (refs. 1–6). Because of this situation it was decided to re-investigate this problem in connexion with our work on the induction of callus growth in bean stem sections by 2,4-D (ref. 7).

The details of the preparation and isolation of the radioactive, water-soluble products from bean stems which were treated with carboxyl-^{14}C-2,4-D will be reported elsewhere. Briefly, bean stems were vacuum infiltrated with 2 per cent sucrose and 10^{-4} M carboxyl-^{14}C-2,4-D (2 mc./mM), surface sterilized by brief immersion in 0·5 per cent sodium hypochlorite solution, rinsed in sterile distilled water, and incubated in nutrient medium for 2 days. They were lyophilized, pulverized, and continuously extracted with wet acetonitrile. After removal of the solvent by distillation, ether-soluble material was extracted at *p*H 1·5, and sedimenting insoluble matter discarded. The aqueous layer was chromatographed on a 'Dowex-1' formate column using a gradient of increasing formic acid concentration. The radioactive peak was freed from formic acid and further purified by passage through a water-saturated silicic acid column using water-saturated 35 per cent *n*-butanol in chloroform as the eluting agent. The product was homogeneous by paper chromatography in the solvent of Jaworski *et al.*[4] and contained no detectable ninhydrin-positive contaminants.

When this material was absorbed on to a small amount of charcoal ('Nuchar') in a sintered glass funnel, the elution pattern shown in Table 1 was obtained. Separate re-application of each of these four fractions to fresh charcoal resulted in their elution at the same relative positions. When another aliquot of the purified material was applied to a charcoal column which differed from that described by Stambaugh and Wilson[8] only in that the column was doubled in length while column diameter and volume of eluting solvent were halved, the distribution of radioactivity shown in Fig. 1 was obtained. This pattern was very reproducible and suggests the presence of some 10 different components in this mixture. In practice, it was found that not all these materials were equally stable, and if great care was not taken during the chromatography on 'Dowex-1', there was considerable hydrolysis, resulting in the release of some radioactivity in ether-soluble form accompanied by the appearance of at least 10 distinct ninhydrin-positive materials in the water-phase. The remaining water-soluble radioactivity was eluted from charcoal only with 1 per cent ammonium in 25 per cent ethanol.

Table 1. ELUTION OF PURIFIED RADIOACTIVE, WATER-SOLUBLE AND ETHER-INSOLUBLE BEAN EXTRACT FROM 'NUCHAR'

A total of 3,000 c.p.m. were applied to 0·5 gm. charcoal. Three 10-ml. fractions were collected with each solvent

Solvent	Total c.p.m. eluted
Water	144
25 per cent ethanol (aqueous)	763
1 per cent NH_4OH in 25 per cent aqueous ethanol	1,496
50 per cent aqueous acetonitrile	306
Total recovered	2,709

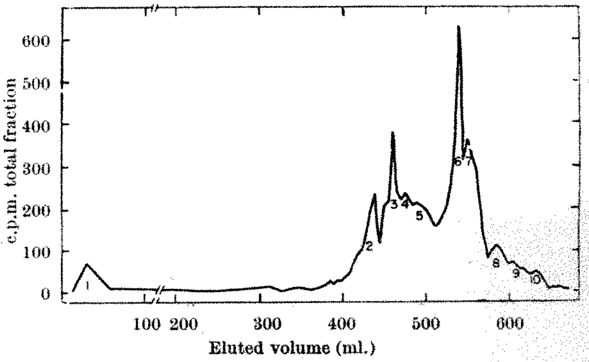

Fig. 1. The elution of radioactivity from 12-in. charcoal columns. 5 ml. fractions were cut between 350 and 700 ml. eluted volume

Holley[2] suggested that the ether-soluble hydrolysis product of the water-soluble bean fraction might be 6-OH-2,4-D, even though the compound was unknown at that time. Since then, 6-OH-2,4-D has been prepared by Cavill and Ford[9]. Using a small sample of this material, generously supplied by Dr. D. L. Ford, we found that it travels on paper indistinguishably from 2,4-D in the solvents previously used. However, when methylethylketone–conc. ammonium–water (100 : 5 : 3·2 v/v/v) was used the two compounds were readily separated. In this solvent the ether-soluble radioactive hydrolysis product from the bean extract was clearly separated from both these known substances (R_F: 2,4-D = 0·35; 6-OH-2,4-D = 0·61; bean extract hydrolysate = 0·0). Counter-current distribution of the ether-soluble material at *p*H 5·1 between phosphate buffer and diethylether resulted in the separation of four radioactive components. These results suggest that the previously reported main product of 2,4-D metabolism in beans[1–4] is indeed a complex mixture of amino-acid amides of several different and as yet unknown acid metabolites of 2,4-D. Further work towards the identification of these acids is in progress.

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Excretion and Hepatic Storage of 5,6-Dimethylbenziminazolyl Cobamide Coenzyme labelled with Cobalt-58

Barker *et al.*[1,2] recently isolated a number of vitamin B_{12} derivatives from bacteria and demonstrated that they act as coenzymes in the conversion of glutamate to β-methylaspartate. Since the 5,6-dimethylbenziminazolyl cobamide coenzyme (DCC) was found to occur in rabbit liver[2], and to act as a co-factor in a rat enzyme system[3], it is reasonable to assume that the coenzymes are the forms in which vitamin B_{12} exists in the tissues and that the 'extrinsic factor' may largely consist of DCC and similar compounds. Since we have previously been engaged in research on the turnover of vitamin B_{12} (refs. 4 and 5), we thought it of interest to perform similar work on the turnover of radioactive DCC in man.

Four healthy student nurses who volunteered for the experiment were given an intragluteal injection of DCC labelled with cobalt-58. The preparation was kindly provided by the Squibb Institute for Medical Research, New Brunswick, New Jersey, and had a specific activity of about 1 μc. per μgm. The radioactive DCC was injected soon after its arrival from abroad and protected from light in order to minimize breakdown.

Three of the test subjects received a dose of 1 μgm. and one 0·75 μgm. in 1 ml. of physiological saline. The test subjects collected their excreta in one-day portions for 39 days (except on days off), and later fæces only during the seventieth to seventy-ninth day following the injection. The samples of fæces were incinerated at 600–800° C. in porcelain cups in a coal furnace in the boiler room of the hospital. Recovery experiments demonstrated that no loss of radioactivity occurred during this procedure. The urine samples were concentrated by evaporation. The ashes and the concentrates were counted in a well-type scintillation counter together with appropriate standards, and the radioactivity was expressed as mμgm. of the injected DCC.

Radioactivity counts were also taken over the liver area under standard conditions, once a day for the first 39 days, and once on the sixty-fifth day. The counts were corrected for radioactive decay.

The results of the excretion work are given in Table 1. It is seen that during the first few days after the injection the urinary excretion was slightly elevated, but later on the excretion of radioactivity was remarkably constant. From the fifth day on, urinary and fæcal excretions were about equal. The fraction of the total-body radioactivity lost per day was about 0·3 per cent or slightly less. This value corresponds to a biological half-life of about one year.

From the first day following the injection, relatively strong radioactivity was measured over the liver area, the counts ranging from 246 to 828 c.p.m. above the background ($\sim$410 c.p.m.). Maximum counts were observed within the first 12 days. During the 65-day period when liver counts were taken, there was no clear-cut decrease in hepatic radioactivity. Thus the hepatic biological half-life must be long.

On the whole, the results obtained with radioactive DCC are very similar to those observed after administration of radioactive vitamin B_{12}[4,6-8]. When radioactive DCC is injected there is possibly slightly less 'spill-over' of radioactivity into the urine in the days immediately following the injection. During later periods the excretion-rates and the total-body biological half-lives of the two compounds are very similar.

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HÆMATOLOGY

Occurrence of Heparin in the Blood of Fish and its Prevention of the Clotting of Fish Plasma

The property of the blood of fish to clot rapidly provides a means of protection from fatal hæmorrhage such as may follow injuries[1]. Sometimes in the same fish the blood clotting-time is known to differ markedly at two different phases of its life-cycle. In the pre-spawning phase when the fish is fully laden with reserves, blood clotting is almost instantaneous, whereas during the period of physiological stress which generally follows spawning, clotting is slow and prolonged. Such variations in the coagulation-time have been attributed to some unknown factors which may involve a change in the chemistry of the blood of fishes[2].

Table 1. MEAN DAILY EXCRETION OF RADIOACTIVITY

The radioactivity is expressed as mμgm. of the injected preparation. *F*, fæces; *U*, urine; %, sum of fæcal and urinary excretions expressed as percentage of the radioactivity estimated to be contained in the body at the time of excretion.

Case Injected dose	*A* 1 μgm.			*B* 1 μgm.			*C* 1 μgm.			*D* 0·75 μgm.		
	F	*U*	%	*F*	*U*	%	*F*	*U*	%	*F*	*U*	%
1st–4th day	0·4	6·2	0·68	2·2	5·1	0·75	0·3	5·9	0·64	0·6	3·7	0·59
5th–39th day	1·5	1·1	0·28	1·0	1·4	0·26	1·0	1·4	0·26	1·1	1·1	0·32
70th–79th day	1·3	1·0*	0·29	1·2	1·7*	0·38	—	—	—	0·7	0·7*	0·24

* Because quantitative collection of urine for long periods is difficult, the subjects collected only fæces during this period. The urinary excretion was estimated on the assumption that the *U* : *F* ratio was the same as during the preceding collection period.

Heparin, the well-known anticoagulant, is a polysulphuric acid ester of a polysaccharide[3]. It has the remarkable property of preserving the fluidity of blood both *in vitro* and *in vivo*[4]; and for this reason in recent years there has been increasing recognition of its value in medicine and surgery[5]. The presence of heparin has generally been recorded in the blood and tissues of homoiothermous animals such as mammals which form the chief source for its extraction[6]. In poikilothermous animals and more particularly in fishes there appears to be no previous account of its occurrence and mode of action.

During a study of the hæmatology of the Indian freshwater murrel *Ophicephalus punctatus* (Bloch), blood samples were collected with a hypodermic syringe from the truncus arteriosus after pithing the fishes and exposing the heart. It then appeared that the clotting time of blood was short and that without the use of anticoagulants, suitable samples could not be obtained. A solution of peptone, as used by earlier workers in higher animals[7], was injected to liberate excessive quantities of heparin *in vivo*. This gave satisfactory results as the blood lost the power of clotting temporarily although the peptone solution in itself did not prevent clotting *in vitro*. It thus became evident that even in fishes production of heparin in order to maintain the fluid nature of blood was a natural phenomenon. Further tests were carried out to illustrate the presence of heparin in blood and its capacity to prevent the clotting of plasma.

Table 1

	Species : *Ophicephalus punctatus*		
	Normal	2 hr. after peptone injection	2 hr. after heparin injection
Prothrombin time at 37° C.	29·4 sec.	58·32 sec.	4 min. 42·5 sec.
Test with toluidine blue	Faintly positive	Positive	Strongly positive

Table 1 gives the plasma prothrombin time of *O. punctatus* by using standard methods[8] under the different conditions. The results indicate that the prothrombin time was prolonged after injecting 0·5 ml. of 5 per cent peptone solution per 100 gm. body-weight. It was considerably longer when the fishes were heparinized with 0·1 ml., heparin B.P., 1,000 I.U. per ml. for every 100 gm. body-weight. In both these cases intramuscular injections were given on the back. Each value given in Table 1 was obtained by using 10 individuals which each gave 2–3 readings. Tests with toluidine blue, as have been recommended for heparin[9], also gave positive results (Table 1).

It therefore seems certain that this remarkable anticoagulant occurs in the blood and possibly in other fish tissues also. It would be interesting to have more information about the piscine mast cells and their occurrence in various tissues, for these cells in other animals are known to secrete heparin[10]. The mechanism of action of heparin in fishes seems similar to that in higher animals[11]. It partly prevents the conversion of prothrombin to thrombin and partly antagonizes the action of thrombin itself. The co-factors such as plasma protein, serum albumin or heparin complement required for the action of heparin seem to be present in fish plasma.

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A New Hæmoprotein with a Unique Iron Porphyrin Prosthetic Group isolated from Human Erythrocytes

Hæmoglobin, comprising more than 95 per cent of the protein of the red cell, is the major protein in the mature human erythrocyte. The extremely high concentration of this compound in the red cell renders very difficult the identification of other hæmoproteins in the erythrocyte. Catalase is one hæmoprotein which has been isolated from erythrocytes. A third hæmoprotein, spectrally similar to hæmoglobin but which has been reported to be functional in the methæmoglobin reductase system, has also been reported[1,2]. It is the purpose of this communication to present observations on still another hæmoprotein which has been isolated from normal human erythrocytes. This compound is particularly noteworthy since its prosthetic group appears to be different from any previously reported naturally occurring iron porphyrin.

This hæmoprotein can be readily distinguished from hæmoglobin or catalase by its spectral properties as shown in Fig. 1. In its oxidized form the compound has no sharp absorption peaks in the visible region while in the Soret region of the spectra it has a typical hæmoprotein absorption with a maximum at 416 mμ. The cyanide derivative of this compound has a broad peak in the visible region with a maximum at 560 mμ and in the Soret region the peak is at 424 mμ. In contrast to catalase, the new hæmoprotein can readily be reduced with dithionite. The reduced form has absorption maxima at 560 mμ and 434 mμ.

Table 1 compares the position of the absorption maxima of the hæmoproteins known to occur in the red cell. A clear distinction can be made between

Table 1

Hæmoprotein	Oxidized	Absorption maxima		
		Cyanide	Reduced	CO reduced
Preparation	416	424	434	422
Catalase (ref. 6)	407	425	—	—
Myeloperoxidase (ref. 7)	430	458	475	—
Methæmoglobin	405	415	430	418

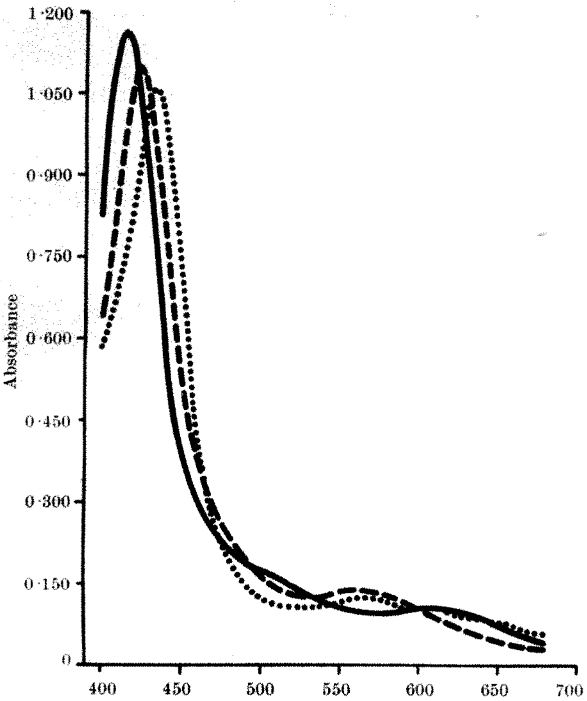

Fig. 1. ——, The hæmoprotein is dissolved in 0·1 *M tris*(hydroxymethyl) aminomethane buffer *p*H 7·2. The solution contained 2·2 mgm. protein per ml.; . . . . , the same preparation as described above to which has been added a few crystals of solid $Na_2S_2O_4$; – – –, the hæmoprotein preparation dissolved in 0·1 *M tris*(hydroxymethyl) aminomethane containing 0·01 *M* cyanide

the newly isolated hæmoprotein and catalase or hæmoglobin. It is also easily distinguished from myeloperoxidase, which might conceivably have been present if the white cells had not been completely removed in preliminary stages of the preparation.

The iron porphyrin prosthetic group of this compound appears to differ from that reported for any other hæmoprotein. Treatment of the preparation with acidified acetone as previously described[3] does not yield a hæmin which is extractable by ether. However, addition of pyridine and alkali to the hæmoprotein preparation does yield a hæmochromogen spectra. The reduced hæmochromogen has an α-peak at 572 mμ, β-peak at 535 mμ and γ-peak at 430 mμ.

A purified preparation of the hæmoprotein was analysed for protein by the biuret procedure[4] and the iron content was analysed as previously described[5]. Assuming that each molecule contains a single iron porphyrin moiety, the molecular weight was calculated to be approximately 67,500.

The hæmoprotein preparation did not appear to act as a peroxidase. No peroxidase activity could be attributed to the hæmoprotein when guiacol, dihydroxyphenylalanine or O-dianisidine were employed as hydrogen donors.

The preparation did not react with reduced di- or tri-phosphopyridine nucleotide. However, the hæmoprotein could be reduced by a highly purified reduced triphosphopyridine nucleotide diaphorase (Morrison, M., unpublished work), which was also prepared from the red cells. In enzymatic properties the diaphorase appears to be similar to the methæmoglobin reductase reported by Huennekens *et al.*[1,2] and required methylene blue in order to reduce the hæmoprotein. This suggests that the hæmoprotein may play some part in electron transport. However, the compound does not have the typical hæmochromogen spectra of a cytochrome. It combines with both carbon monoxide and cyanide, which indicates that if its does play a part in electron transport it may act as an oxidase.

The method of preparation and a more detailed account of the characterization of this hæmoprotein will be published elsewhere.

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HISTOLOGY

Mast Cells in Peripheral Nerve Trunks

References to mast cells occur only rarely in histological accounts of the structure of peripheral nerve trunks. They were mentioned by Cajal[1] and by Riley and West[2] in the perineurium of the rabbit and of the rat respectively, and in the looser connective tissues of the epineurium; they have been described in the endoneurium in the bony fish *Orthagoriscus mola*[3]. In injured nerve trunks, however, Cajal[1] has figured and briefly mentioned mast cells as elements of the endoneurium of the rabbit.

Recently we have studied thin (about 1μ) sections of rat sural and caudal nerves, stained with methylene blue after fixation in Dalton's fluid and embedding in 'Araldite' (a technique recommended to us by Dr. K. C. Richardson). Myelin sheaths, Schwann nuclei, etc., were stained as expected, but, in addition, large cells with metachromatically stained granules were frequently seen in the endoneurium as well as in the perineurium and the epineurium. Metachromatic granules or amorphous material frequently extended from these cells into the nearby tissue spaces between adjacent nerve fibres and as an inner lining to the 'endothelium' of the perineurium. Large metachromatically staining cells of this kind are also conspicuous elements in similarly fixed and stained longitudinal (10μ) sections of cat femoral nerve and mouse sciatic nerve. In the guinea pig sural nerve and in the splanchnic nerves of the rat and man they are also present but in smaller numbers. They are often seen in contact with small nutrient blood vessels, but many occur freely in the endoneurial tissues. In the rat nerves they constitute approximately 1·5–2 per cent of all the cells enclosed within the endothelium of the perineurium. They very closely resemble the mast cells that may be demonstrated in the rat's mesentery by similar fixation and staining or by alcohol fixation and staining with methylene blue or toluidine blue. For this reason we consider these metachromatically staining cells to be mast cells.

Recent work[4] suggests that some mast cells may be distinguished by their ability to release adrenaline and noradrenaline when suitably stimulated and may be classified as chromaffin cells. The mast cells of rat sural and caudal nerves do not exhibit the 'chromaffin' reaction as this has recently been defined[5], that is, they do not exhibit a yellow coloration after treatment with Orth's fluid. Subsequent staining of the same sections with methylene blue, however, revealed the normal complement of metachromatically stained cells. At the same time, Orth-fixed suprarenal medulla did show the 'chromaffin' reaction in cells which, when stained with methylene blue, did not exhibit metachromasia.

In injured rat nerves some changes occur in the mast cell population, as compared with normal nerves. In nerves cut 1–8 days previously a higher proportion (statistically significant) of the cells appeared to have shed some or many of their granules into the nearby tissue spaces, although the total number present had not changed. In four nerves injured 8 months previously, however, the number of mast cells present had increased about 4 times; in 5 mm. of rat caudal nerve cut, or crushed and allowed to regenerate, as many as 1,250 were counted, as compared with 250–300 in a similar length of normal nerve.

The function of these endoneurial mast cells is at present obscure. In many peripheral nerves a high content of histamine has been recorded[6], and in other tissues this has usually been related to a high content of mast cells[7]. There is also much evidence to implicate mast cells in the elaboration of connective tissue ground substance and, indirectly, of fibrils, although by means as yet unknown[8]. Thus, while mast cell hyperplasia is marked in conditions of fibroplasia, the latter usually appears first. This observation is consistent with the finding of mast cell hyperplasia only in nerves which have degenerated for a long time, where it may be related to the increase in connective tissue collagen reported by Abercrombie and Johnson[9] in such nerves. Mast cells may play some part in the metabolism of endoneurial connective tissue, aiding the formation of fibrils but unable to initiate the process. Their presence may be relevant to the problem of the origin of this tissue, which is by no means satisfactorily solved[10].

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PATHOLOGY

Histochemical Evidence of 5-Hydroxytryptamine in a Dog Mast Cell Tumour

Mast cells of dogs are said not to contain 5-hydroxytryptamine[1–4]. Furthermore, this substance has not been found in neoplasms of these cells in dogs[5]. An instance of a dog mast cell tumour is reported here, however, in which there was histochemical evidence of the presence of 5-hydroxytryptamine.

The tumour removed surgically was a cylindrical teat-like projection from the abdominal surface of a black terrier. The neoplasm measured 3·5 cm. × 1·8 cm. × 1·3 cm. It was of soft texture and grey-white in colour. It had the characteristic histological structure and cytological features of a mast cell neoplasm. The tissue was fixed in acid (*p*H 5·5) formalin and examined histochemically. A toluidine blue stain revealed clusters of large round cells having the characteristic metachromatic granules of mast cells (Fig. 1). The histochemical tests for 5-hydroxytryptamine have been devised to distinguish this substance from other related compounds.

Schmorl's ferric ferricyanide produced a strong reaction, indicating the presence of reducing substances in the granules which had survived formalin denaturation; catechols do not[6].

Black cytoplasmic granules in most of the mast cells of the tumour following Lillie's diazo-safranin reaction indicated the presence of enterochromaffin substance. The identity or close chemical relationship of enterochromaffin substance and 5-hydroxytryptamine is well established. Lillie claims that phenylalanine, tyrosine, tyramine, tryptophan and other tryptamines all fail to produce this reaction with this reagent[6].

Frozen sections of the material fixed in acid formalin were examined by ultra-violet light emitted from a General Electric *AH*6 1,000-watt mercury arc, and filtered through a Corning heat-absorbing blue filter and a Corning ultra-violet-transmitting filter (No. 5840) with maximum transmission at 350 mμ.

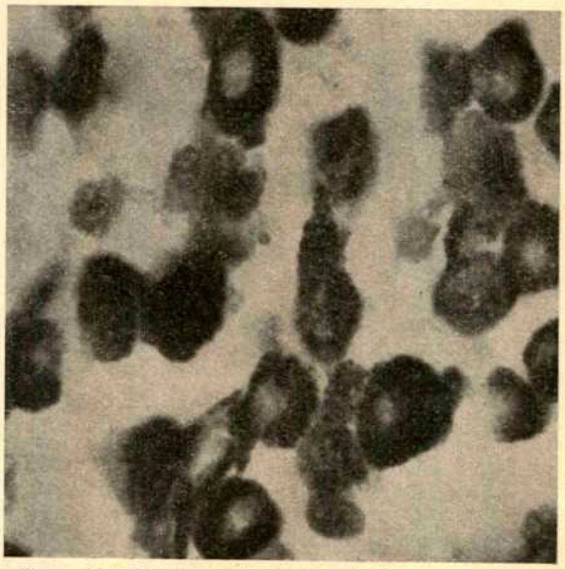

Fig. 1. Mast cells in a frozen section of a dog neoplasm stained with toluidine blue. The cytoplasmic granules which were strongly metachromatic are prominent

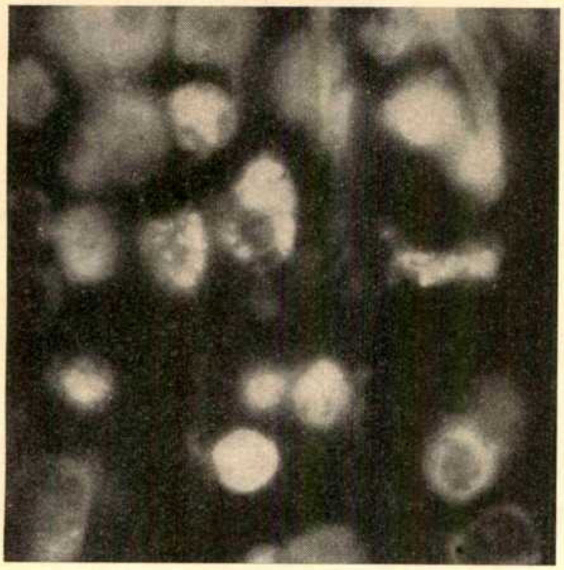

Fig. 2. Bright (orange) fluorescence in granules and in cytoplasm of the dog mast cells treated with Benditt's ninhydrin–acetic acid reaction

A Reichert Zetopan microscope equipped with an ultra-violet-transmitting dark-field condenser was used for the observations. The cytoplasmic granules of most, but not all, of the mast cells exhibited a bright golden-yellow fluorescence. The fluorescence was absent or imperceptible when a mercury arc of lower intensity (Osram 200) was used. Benditt modified Jepson and Stevens's ninhydrin method for paper chromatography[7] so that it can be used histochemically to demonstrate fluorescence of tryptamines[8]. Ninhydrin is sublimed (at 5×10^{-3} mm. mercury at 100–120° C.) on to the section of tissue which is then treated with vapours of acetic acid and acetone and examined with ultra-violet light. The mast cells so treated showed discrete, orange, fluorescent cytoplasmic globules (Fig. 2).

The following is a summary of the histochemical evidence of 5-hydroxytryptamine in the mast cell tumour :

Histochemical procedure	Results
Toluidine blue	Metachromasia of cytoplasmic granules (Fig. 1)
Schmorl's ferric ferricyanide	Strong reaction
Gomori's diazonium reaction	Strong reaction
Lillie's diazo-safranin for enterochromaffin	Strong reaction
Ultra-violet fluorescence	Golden-yellow fluorescent granules
Vapour-phase ninhydrin (for tryptamine derivatives)	Orange fluorescent granules (Fig. 2)
Methenamine–silver	Variable

According to Benditt (ref. 9 and personal communication from Benditt, E. P., to W. G. R), and Barter and Pearse[10], a specific fluorescence is produced by the interaction of acid formalin and 5-hydroxytryptamine. Catechols and resorcinol do not fluoresce like that after formalin treatment. The fluorescence noted in enterochromaffin cells after acid–formalin fixation is concluded to be due to 5-hydroxytryptamine.

This fluorescence along with positive ferric-ferricyanide and diazo-safranin (Lillie) reactions are strong presumptive evidence of the occurrence of 5-hydroxytryptamine in the mast cells of this tumour.

The variable methenamine–silver reaction cannot be equated with 5-hydroxytryptamine production[11] ; therefore, the failure consistently to reduce silver in this case does not indicate its absence.

Histochemical evidence of the presence of 5-hydroxytryptamine in a dog mast cell tumour implies an intraspecies as well as interspecies difference in its distribution in these cells. When induced ultra-violet fluorescence is being studied, the importance of using a high-intensity light source is emphasized.

This work was supported by U.S. Public Health Services research grant C–4521, and a training grant 2–G–207.

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Identification of So-called Atypical Macroglobulinæmia as an Atypical Form of β_{2A}-Paraproteinæmia : a New Immunological Entity?

OCCASIONALLY, ultracentrifugation of sera from patients with multiple myeloma yields an abnormal protein fraction with a sedimentation constant of 8–12 *S*. Jahnke and Scholtan[1] referred to such cases as "atypical macroglobulinæmia", because the sedimentation constant of these pathological proteins is smaller than that of the 'true' macroglobulins such as found in Waldenström's macroglobulinæmia.

Little is known as yet about the nature of these proteins. It was pointed out by Putnam[2] that these rapidly sedimentating proteins are exclusively encountered in the case of so-called β-myelomata. They differ from the β_{2M}-macroglobulins in immunological characteristics[3].

In sera from nine patients suffering from multiple myeloma, investigation with the aid of the ultracentrifuge revealed an abnormal protein fraction with a sedimentation constant between 8 and 12. In most cases, this rapidly sedimenting component was visible in the ultracentrifuge diagram as a sharp peak with a narrow base—a picture characteristic of paraproteinæmia.

Sulphydryl reagents did not dissociate these atypical macroglobulins into smaller 6-*S* fragments, a fact which is in contrast with the findings obtained in Waldenström's macroglobulinæmia[4]. Disulphide bridges, therefore, are probably of no importance in the macromolecular structures of the proteins under investigation.

After electrophoresis in agar-gel[5], a pathological protein fraction in the β-globulin or high-speed γ-globulin region was found in all sera (Fig. 1*a*). Two abnormal protein fractions were present in two cases. The quantitatively most important fraction was found in the β-globulin region, while the less pronounced component was localized in the γ_2-globulin area (Table 1). All paraprotein fractions were periodic acid–Schiff-positive, but failed to stain with sudan black.

Two sera (*Be* and *Bu*) were examined by zone electrophoresis on starch. It was found that the paraprotein component of both sera was separated into two fractions, which could be isolated. The chemical and immunological analysis of these fractions is now in progress.

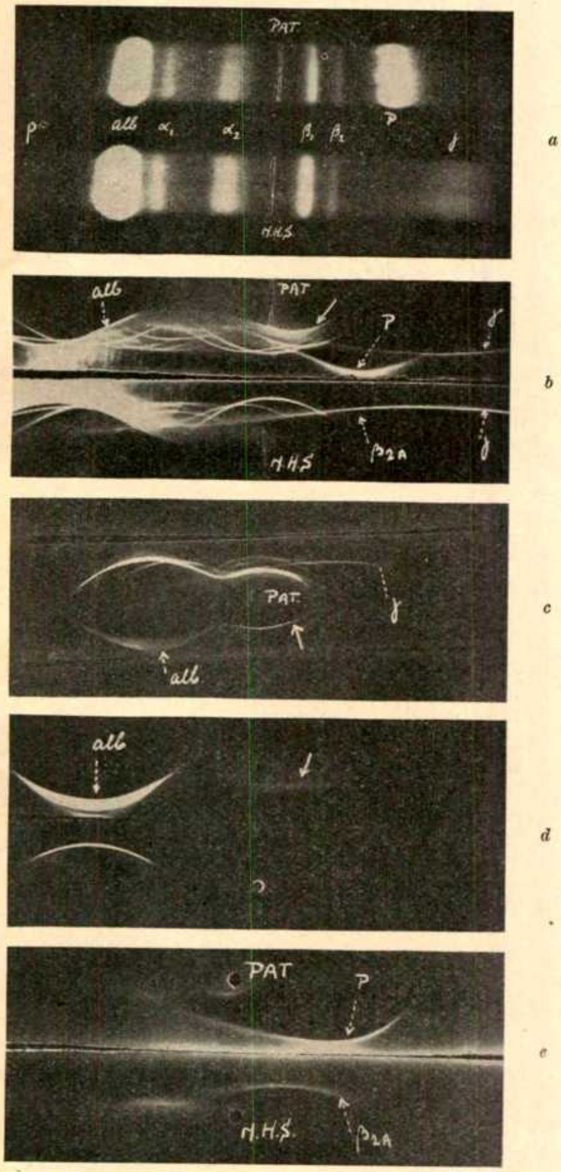

Fig. 1. *a*, Pherograms (agar gel-electrophoresis; barbital buffer *p*H 8·6; 35 V./cm.) obtained on normal human serum (*N.H.S.*) and on the serum of patient *Bu* (*PAT*). Note the paraprotein fraction in the latter serum (*P*). *b*, Immuno-electrophoresis of the serum of patient *Bu* (upper basin) and of normal human serum (lower basin). Note the deformation in the β_{2A}-line (*P*) and the abnormal albumin precipitation line (arrow). The antiserum used was *K*6. *c*, Immuno-electrophoresis of the serum of patient *Ve*. The antisera used were *K*6 (upper basin) and anti-human albumin immune serum (lower basin). *d*, Immuno-electrophoretic analysis of the serum of patient *Be* before (upper basin) and after (lower basin) treatment with cysteine. The antiserum used was anti-human albumin immune serum. Note the disappearance of the albumin line in the β-globulin region. *e*, Immuno-electrophoresis of the serum of patient *Bu* (upper basin) and of normal human serum (lower basin). The antiserum was *K*6, absorbed with umbilical cord serum and normal human serum. It is clearly demonstrated that the paraprotein fraction has β_{2A}-antigenic determinants

Table 1

Patient	m_r	Localization in the pherogram
Be	0·31	Between β_2 and γ-globulin
Bon	—	Dense precipitate behind application groove
Bo	0·35	Just behind the β_2-globulin
Bu	0·28	In the fast γ-globulin region
De	0·29	In the fast γ-globulin region
Le	0·29	In the fast γ-globulin region
PB (main fraction)	0·30	Between β_2- and γ-globulin
(small fraction)	0·12	In the slow γ-globulin region
Pu (main fraction)	0·39	On the β_2-globulin fraction
(small fraction)	0·27	In the fast γ-globulin region
Ve	0·41	On the β_2-globulin fraction
β_2-globulin in normal human serum	0·42	

Relative mobilities m_r of the paraprotein fractions in the serum of 9 patients with 'atypical macroglobulinæmia'. Agar-gel electrophoresis. Difco 'Nobel Agar'. Barbital buffer *p*H 8·6; voltage 35 V./cm. For technical details see ref. 5.

For the purpose of more precise characterization of the paraprotein fractions in the sera from our patients, these sera were submitted to immuno-electrophoretic examination. With the aid of a horse antihuman immune serum (Institut Pasteur, Paris), we obtained results such as those seen in the case of β_{2A}-myelomata[6,7]. The β_{2A}-line in the diagrams was considerably accentuated and displaced towards the antiserum reservoir (Fig. 1*b*). The β_{2M}-line was as a rule lacking. The γ-line sometimes showed shortening towards the anode side. The same results were found with a rabbit immune serum (*K*6) against the serum of one of our patients (*Be*). In the β-globulin region there was in the immuno-electrophoretic examination of all sera a short but heavy precipitation line, situated near the antigen-cup. This abnormal line, which could not be detected in immuno-electrophoretic analysis of normal human serum, shows a reaction of identity with the albumin line (Fig. 1*b*—arrow). This was clearly demonstrated by immuno-electrophoresis of the patients' sera with a rabbit anti-human albumin immune serum (Behringwerke) (Fig. 1*c*).

The abnormal protein fraction in the β-globulin region is probably a complex of albumin with a yet unknown protein. This complex could be dissociated by treatment with cysteine (Fig. 1*d*).

These findings have only been reported before by Heremans[8]. In some of the cases of β_{2A}-myeloma, investigated by him, this albumin complex in the β-globulin region was found. It is likely that these cases represent β_{2A}-paraproteinæmia with a rapidly sedimentating protein component.

Besides this abnormal albumin precipitation line there was sometimes additional abnormal precipitation lines in the β-globulin region. By immuno-electrophoresis with a rabbit anti-human γ-globulin immune serum, it was demonstrated that these lines have a γ-globulin antigenic structure.

All sera were examined by immuno-electrophoresis, with a specific rabbit anti-β_{2A}-globulin immune serum (Behringwerke) as anti-serum. In all cases, a precipitation line was visible which corresponded with the line *P* found with horse antihuman immune serum; this proved that the paraproteins under investigation did in fact possess β_{2A}-antigenic determinants.

A rabbit anti-serum (*K*6) was prepared against the serum from patient *Be*. This anti-*Be* immune serum was absorbed with umbilical cord serum and normal human serum. After absorption, this antiserum contained only antibodies against β_2A-globulins because—both in immuno-electrophoresis with normal human serum and with the sera from our patients—it reacted by forming the β_2A-line (Fig. 1*e*). Cross-reactions were excluded with certainty because, using Ouchterlony's agar diffusion technique, reactions of identity were obtained between the sera from our patients, both with rabbit anti-β_2A-globulin immune serum and with absorbed rabbit anti-*Be* immune serum.

A β_2A-paraproteinæmia with the presence of an abnormal albumin component in the β-globulin region was found to exist in the sera from nine multiple myeloma patients examined, containing an abnormally rapid sedimenting ($s_{20} = 8 - 12\ S$) protein fraction. If this is to be found in all cases of so-called atypical macroglobulinæmia, then it would be advisable to abandon the designation 'atypical macroglobulinæmia' (which is incorrect because it suggests a non-existing correlation with Waldenström's macroglobulinæmia[9-11]) in favour of the term 'atypical β_2A-myeloma'.

We wish to thank Dr. P. Burtin of the Institut Pasteur, Paris, for analysing two sera, and Dr. G. Schwick of the Behringwerke for supplying rabbit anti-β_2A-globulin immune serum.

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Fibrosarcoma in a Rabbit following β-Irradiation of the Skin

THIS communication describes the gross and histological appearance of a fibrosarcoma which occurred four years after exposing an area of rabbit skin to 16,000 rads from a phosphorus-32 plaque. The only previous observation of skin tumours in rabbits resulting from radiation exposure is contained in a recent communication by Sissons and Vaughan[1]. These workers described squamous cell carcinomas in the external auditory meatus of rabbits injected with strontium-90 at an early age. The available literature indicates a low natural incidence of tumours in rabbits[2,3].

Four years ago, we began work in this Laboratory to determine the variation in the skin reaction of rabbits, pigs and sheep after acute exposure of doses of 2,000, 8,000 and 16,000 rads of β-irradiation. Six rabbits were exposed to either strontium-90 or phosphorus-32 plaques at two sites per animal with four sites exposed to each of the dosage-levels indicated. The radiation intensity was in the range of 5,000–8,000 rads/hr. The experimental animals are being kept for their life-span. The clinical appearance of the acute and chronic reactions is being reported separately (L. A. G. and L. K. B., in preparation).

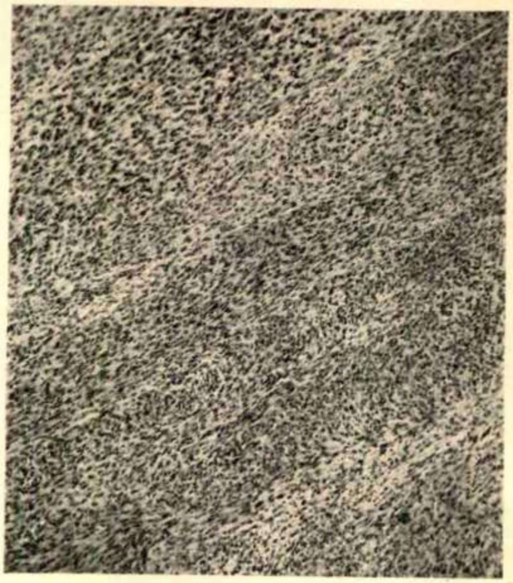

Fig. 1. Bundles of connective tissue fibres in well-differentiated areas of primary skin tumour (× 65)

The primary tumour occurred at the site of the original β-irradiation exposure. The tumour formed a moderately firm, spherical mass, measuring 6 cm. in diameter and raised to a height of 5 cm. above the body surface. The mass was located in the loose connective tissue between the body wall and the skin, which had become necrotic at the irradiation site over the tumour. Metastases were observed

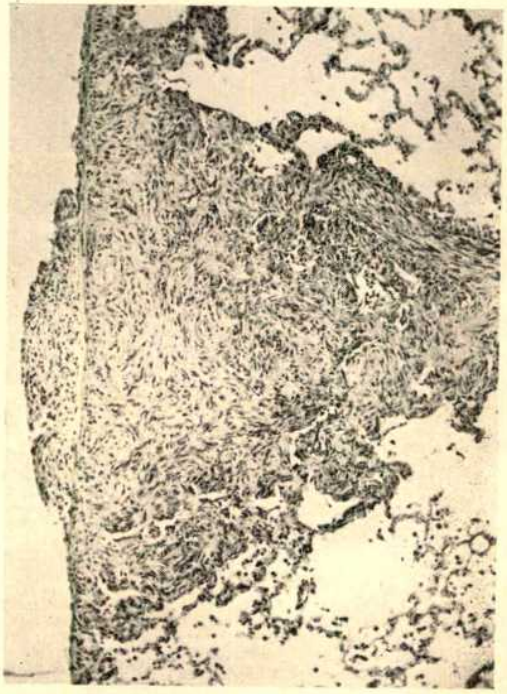

Fig. 2. Pulmonary metastasis. (× 65)

grossly in the lung. The lesions in the lungs consisted of several small (1–2 mm.), round, white nodules on the surface of the right and left diaphragmatic lobes.

The basic histological pattern of the tumour consisted of interlacing bundles of moderately cellular fibrous connective tissue (Fig. 1). The well-differentiated areas showed few mitotic figures. A network of fibres between the individual cells was demonstrated in the differentiated areas with the Laidlaw stain. Other regions of the tumour disclosed moderate pleomorphism with rounding of the nuclei and diminished formation of fibres. Mitoses were somewhat more frequent in such areas, which also contained foci of œdema and necrosis. The pulmonary metastases revealed a relatively loose pattern of fibroblastic cells (Fig. 2). The small metastases were often necrotic except for a narrow peripheral rim of viable tissue. The tumour was diagnosed pathologically as a fibrosarcoma.

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BIOLOGY

Promotion of Leaf Growth by Short Days in the Short-Day Plant *Chenopodium amaranticolor*

PAST investigations into the relationship between length of day, flowering and leaf-growth have failed to explain the complex interactions between them. In most, if not all, plants the development of flower buds is associated with a reduction in leaf-growth and eventually leads to bract formation[1], and Bünning and Konder[2] have shown that leaf growth in plants maintained in a vegetative state is less for inductive than non-inductive conditions in the short- and long-day plants they studied. On the other hand some results obtained by Schwabe[3] seem on re-examination to indicate a promotion of leaf-growth by inductive conditions in *Xanthium*.

Recent work by me (the results of which will be published elsewhere) has shown that conditions leading to flowering in *Chenopodium amaranticolor* initially also promote vegetative growth. Plants which were grown from germination in 16-hr. photoperiods for 26 days were then induced to flower by transfer to various numbers of 8-hr. days. As an immediate result of such transfer the rates of leaf and axillary bud initiation at the apex increased considerably. Four days after the start of short-day treatment the daily rate of leaf initiation had increased to 3·50 from a long-day value of 0·46 and vegetative axillary bud initiation had increased from 0·46 to 2·05 a day. At the same time the rate of growth of all immature leaves was stimulated by exposure to short days, even though it was strongly inhibited afterwards in induced plants. The growth of the leaf at node 7, which reached maturity before the inhibition became dominant, is shown in Fig. 1.

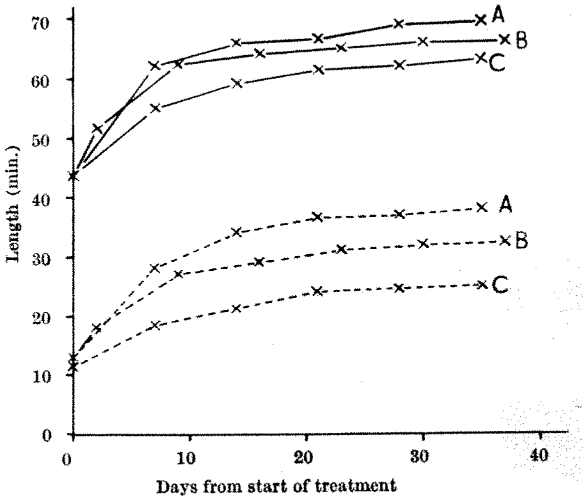

Fig. 1. Effect of inductive treatment on petiole (- - -) and lamina (——) growth of leaf 7 (8 plants per treatment). Plants were grown in long days and then received: *A*, continuous short days; *B*, two short days followed by long days; *C*, long days (control)

To test the closeness of this relationship between leaf-growth and flowering in *C. amaranticolor*, a comparison was made of the effects of short days with and without interrupted dark periods. Plants were grown in a glasshouse in continuous light (consisting of natural daylight in March–April at Palmerston North supplemented by light from 200-W. 'Sieray' mercury vapour lamps ballasted with a tungsten filament which gave an intensity of approximately 500 ft.-candles at the plant apices) for the seven weeks between germination and the start of inductive treatment. They were then divided into four groups of eight as shown in Table 1, 16 receiving 8-hr. photoperiods (–*LB*) and the other 16 receiving 7-hr. photoperiods, with 16-hr. dark periods interrupted at the mid-point by a 1-hr. light break (+*LB*) at an intensity of approximately 3,500 ft.-candles. The total amount of light received daily by each group was the same. Buds were excised from the axils of all leaves longer than 16 mm. immediately prior to short-day treatment. In 16 plants (8 + *LB*, 8 – *LB*) all apical tissue above the youngest leaf longer than 16 mm. was removed and in the other 16 the apical tissue was retained. The effect of these treatments on the growth of the youngest leaf longer than 16 mm. is shown in the table; both with and without apical tissue the growth of the – *LB* groups (flowering) was significantly higher than that of the + *LB* groups (non-flowering). It thus seems very possible that a direct relationship exists between the promotion of leaf growth and the onset of flowering. The fact that a similar relationship occurs in both the presence and absence of apices indicates that the

Table 1. PERCENTAGE INCREASE IN LEAF LENGTH AFTER SIX SHORT DAYS WITH AND WITHOUT INTERRUPTED DARK PERIODS

	– *LB*	+ *LB*	*P* (from *t* test)
+ apex	69·14	53·75	0·019
– apex	77·40	61·25	0·044

growth stimulus is related to some step preceding the initiation of flower primordia and does not emanate from them.

Investigations to determine the universality of the promotion of leaf-growth which occurs in inductive conditions in *Chenopodium* and *Xanthium*[3] are in progress. Meanwhile the situation prevailing at the apex, where the transition to flowering is clearly associated with an increased growth-rate, indicates that such a tendency to increased leaf growth throughout the plant probably occurs very widely concomitant with the onset of flowering. Once flower primordia are formed, however, they exert a strong inhibitory influence on leaf growth which quickly suppresses any initial stimulation which might have been present.

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Estimation of Oil in Safflower Seed

WEIGHT per measured bushel is a widely used index of physical quality of grain[1]. In applying this measure of quality to varieties of safflower (*Carthamus tinctorius*) grown on dry land in southern Alberta during 1957–60, there was a correlation of $r = -0.68$ ($P < 0.01$) between oil percentage and bushel weight. The weight per measured bushel of varieties varied from 36·5 to 49·5 lb., whereas the oil content varied from 37·9 to 25·0 per cent, respectively. Since this relationship is contrary to that generally expected, some of the physical and chemical properties of the seed were investigated. Seed from fourteen varieties grown at three locations were used for this purpose.

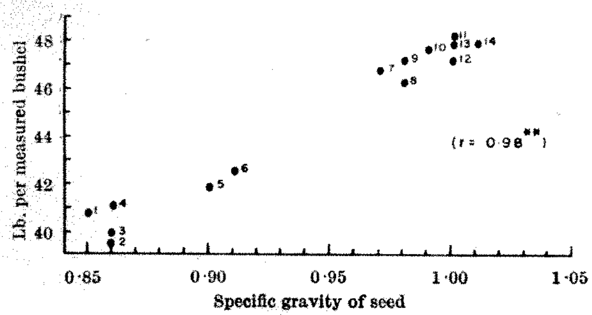

Fig. 1. The interrelationship between oil percentage, weight per measured bushel, and specific gravity of the seed of 14 safflower varieties. $P < 0.01$

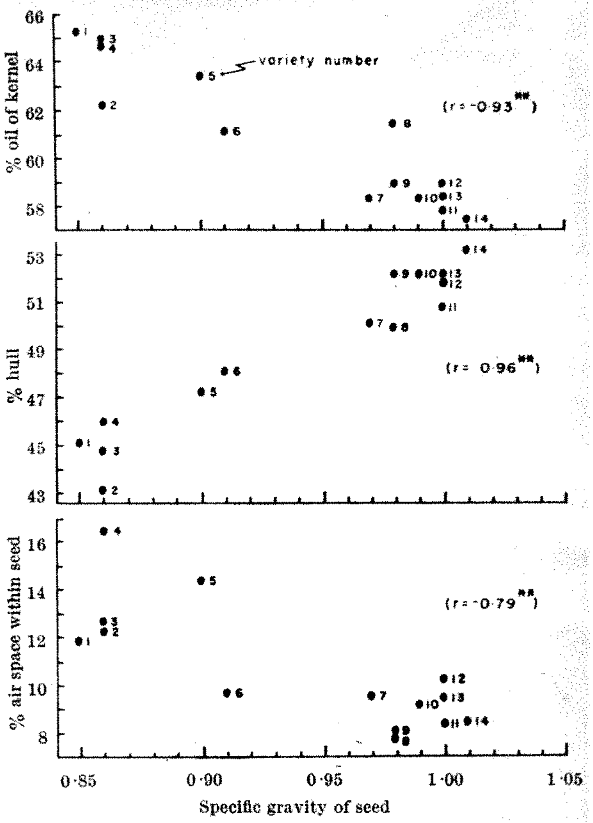

Fig. 2. The interrelationship between specific gravity of safflower seed, air space inside the seed, oil percentage of kernels, and hull percentage. $P < 0.01$

The specific gravity of the seed was determined for each variety on the basis of four 100-seed samples from each location. The varietal means for three locations for oil percentage, specific gravity, and bushel weight are shown in Fig. 1. It is evident from these results (Fig. 1) that varieties with low oil percentages could be eliminated on the basis of either their high bushel weight or high specific gravity. This relationship between oil percentage and specific gravity of seed is sufficiently close to be of practical value in evaluating safflower lines for their oil content. The weight per measured bushel is easily obtained where a substantial quantity of seed is available, but the specific gravity index has the advantage of being equally applicable to both small and large quantities of seed. Furthermore, it can be used to advantage in screening segregating populations.

Since low specific gravity of seed is associated with high oil percentage, additional analyses were carried out to determine some of the factors that might contribute to this relationship. The percentage air space inside the seed was calculated for each variety by determining the difference in volume between whole and split seed. The hull with adhering seed coat was separated from the kernel to calculate the percentage hull of the seed and percentage oil in the kernel. Varietal means of three locations for these determinations are given in Fig. 2. The percentage air space inside the seed and oil percentage of the kernel decreased with increasing specific gravity whereas the hull percentage increased. The association between specific gravity of seed and hull percentage was investigated further by determining the specific gravity of the hulls. Although the specific gravity of the hull was found to vary with varieties, it was always greater than that of the whole seed. This explains the close relationship between specific gravity of seed and percentage hull. The high specific gravity of hull and the factors illustrated in Fig. 2 clarify the relationship between high oil percentage and low specific gravity of safflower seeds.

These results will be reported in greater detail elsewhere.

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Potato Root Eelworm in the Canary Islands

The European and Mediterranean Plant Protection Organisation annual reports 1953–1959, which survey the incidence of the potato root eelworm, *Heterodera rostochiensis*, record the occurrence of this major pest of potatoes in all member countries except Italy, Yugoslavia, Rumania, Turkey, Cyprus, Malta, Libya and Tunisia.

On December 27, 1960, in company with Sr. Iuan Valladares Barbuxano, Ingeniero Agronomo de la Jefatura Agronomica, I discovered the existence of *H. rostochiensis* in potato fields at La Victoria de Acentejo and in other districts in the north of Tenerife, Canary Islands. At latitude 28° 25′ N., this would appear to be the most southerly occurrence as yet recorded in the geographical distribution of *H. rostochiensis* in the Old World.

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A Technique for Destruction of the Pineal Body using Yttrium-90 Spheres

Excision of the pineal body, first described by Exner and Boerse[1], has been frequently used in the study of pineal function and has been performed in various groups of animals[2]. Thermocauterization and stereotaxic ablation have also been employed. The operative mortality with the latter methods has been quite high due to hæmorrhage from adjacent blood vessels[2].

A technique using yttrium-90 spheres for radiological destruction of the pituitary gland was recently introduced[3]. The same source of radiation was used in the present work. The yttrium spheres were manufactured as described by Ginell and Simon[4] and by Ginell and Doering[5]. The activity of the spheres following activation in an atomic reactor was calculated as described by Notter and by Notter *et al.*[3,6]. A 2–3 cm. mid-line incision was made over the occipital region of fifteen rats following anæsthesia which was maintained with ether. A hole about 1·5 cm. in diameter was drilled exactly above the site of the pineal body. The position of the hole had been calculated in relation to the visible sutures between the parietalia and the occipital bone (checked by X-ray photograph, Fig. 1). Three or four spheres were inserted on and around the pineal body. Damage to the venous sinuses, which overlie the gland, could not be avoided but the bleeding induced was easily stopped.

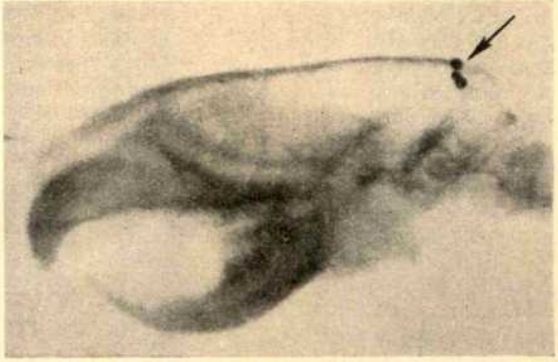

Fig. 1. X-ray photograph of the skull showing the site of the spheres. (× 3)

Four experimental animals died within 2–4 days following the operation, whereas the remaining survived and were killed on the 15th, 20th and 35th day after the operation. The brains and endocrine organs were then fixed for future examination. A preliminary examination of the irradiated tissue of the pineal brain region showed complete necrosis of the pineal body in eight cases. The radiation dose needed to obtain complete necrosis, provided that the spheres were placed correctly in relation to the pineal body, was calculated as approximately 100,000–150,000 rads, thus causing necrosis at the distance of 1·5–2 mm. from the periphery of the spheres.

No appreciable radiation damage was found in the cortex of the brain adjacent to the pineal body,

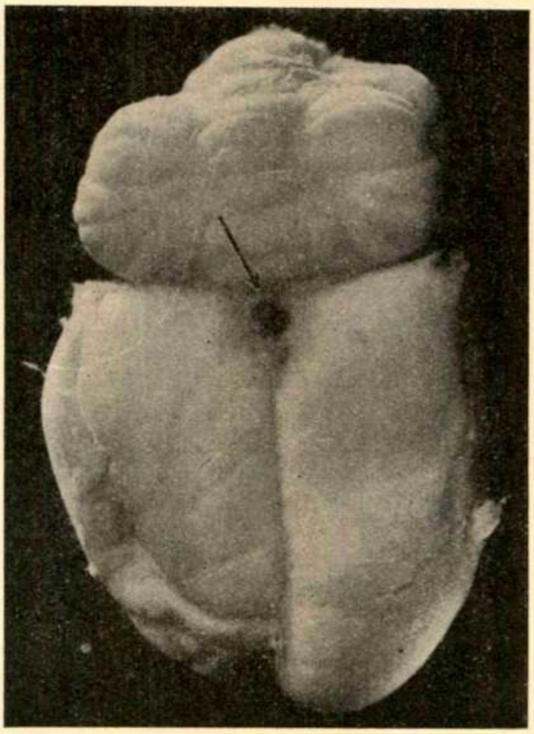

Fig. 2. Dorsal view of the rat brain indicating the position of one of the yttrium spheres. (× 3)

except for two cases in which the spheres had been placed incorrectly in relation to the pineal body. Partial necrosis of the pineal body (50–80 per cent) was obtained in a few cases in which the position of the spheres apparently had not been optimal in relation to the pineal body. However, since a total of eight cases out of 11 surviving cases were obtained with a complete destruction of the pineal body, it was concluded that the method described must be considered as a suitable means for localized destruction of the pineal body, and other small tissue areas as well.

This work was supported in part by the Tullberg Fund, University of Uppsala. We acknowledge the advice of Prof. M. D. Altschule, McLean's Hospital, Waverley, Mass., in preparing the manuscript.

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A Biennial Copepod from Lake Hazen, Ellesmere Island

Several species of copepods in the Arctic are known to have annual life-cycles in the sea[1] and freshwater[2], and macroplanktonic Crustacea of northern seas may not breed until two years old[3]. So far as I am aware, no copepod has been described with a life-cycle greater than one year, and the extreme response of *Cyclops scutifer* in Lake Hazen to conditions of low temperature and limited food seems worth recording.

Lake Hazen (lat. 81° 45′ N., long. 71° 00′ W.) is the largest lake of the Queen Elizabeth Islands of high-arctic Canada. It is glacier-fed, cold and deep, and is not always entirely free from ice in summer. Primary productivity is very low, and the fauna is small in species and individuals.

The zooplankton was sampled regularly between mid-June and mid-August. The proportions of naupliar and copepodite stages at one offshore station on four successive dates are given in Fig. 1. The proportions of the stages were very similar in plankton collections from other parts of the Lake. The conspicuous and persistent bimodality of the population throughout the short sampling period can only be the result of a basically biennial life-history. A very few adults were taken in a surface haul on August 11, the last date of sampling, but presumably most copepodites do not reach adult-hood and breed until the autumn months. The products of this breeding pass the winter as nauplii and just begin to enter the copepodite stages by the following summer. The possibility of slight exchange between the generations is suggested by the stage I copepodites of June 18 and the stage II copepodites of August 11.

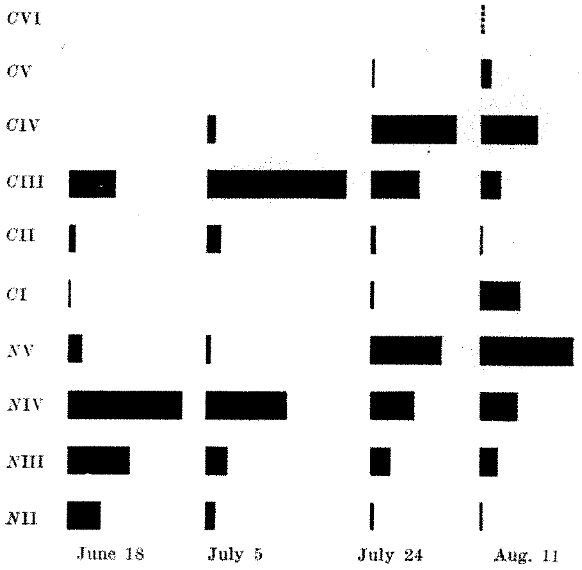

Fig. 1. The proportions of the stages of nauplii and copepodites of *Cyclops scutifer* in vertical plankton hauls from 32 m. at the same station on four successive dates. No adults (*CVI*) were taken in the vertical haul of August 11, but a few were taken in a nearby surface collection on that date

I am indebted to Dr. Edward B. Reed, Colorado State University, for finding the half-dozen adult copepods in the entire collection and identifying the species as *Cyclops scutifer* Sars. Field work was carried out in summer 1958 on the Canadian International Geophysical Year expedition to Ellesmere Island, organized by the Defence Research Board of Canada. Collections were analysed at the Bingham Oceanographic Laboratory, Yale University. A more complete account of the aquatic biology of Lake Hazen is in preparation.

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BACTERIOLOGY

Biosynthesis of Acetoin by *Leuconostoc citrovorum*

It has been shown[1] that in most bacterial cells acetoin is formed by the condensation of pyruvate and activated acetaldehyde. The findings of Beckhorn[2], De Man[3] and Mizuno and Jezeski[4] leave no doubt that in the heterofermentative genus *Leuconostoc* the synthesis occurs via acetolactate. It is still uncertain, however, if acetoin is formed from sugar and, if so, out of which part of the carbon chain of the sugar. Preliminary experiments showed that washed cell suspensions of *Leuconostoc citrovorum* ATCC 8082 do not form acetoin during glucose fermentation in a phosphate buffer. On the other hand, a large amount of acetoin is produced from pyruvate. When pyruvate and glucose are added simultaneously, the synthesis of acetoin is inhibited.

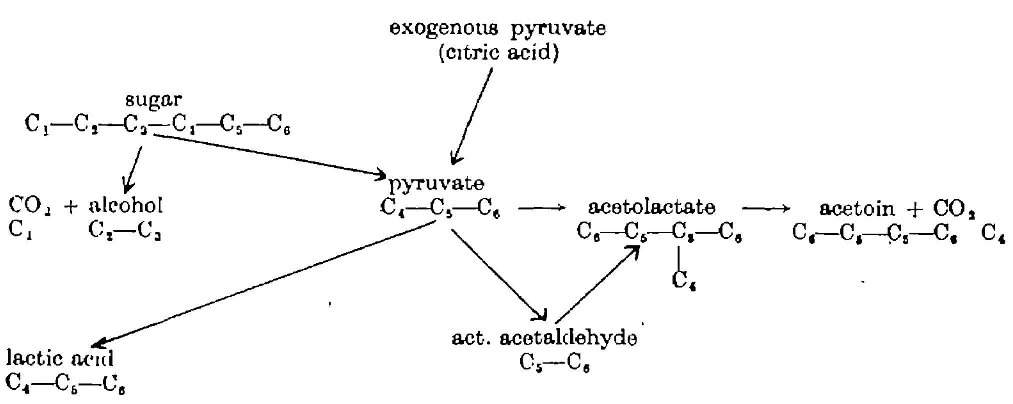

Fig. 1. Scheme of acetoin synthesis by *Leuconostoc citrovorum*

Only at the end of the fermentation, when the glucose concentration becomes so low that the production of carbon dioxide decreases, does the synthesis of acetoin begin. In the presence of a favourable H-donator (glucose) the pyruvate in the cell is presumably reduced so quickly to lactic acid that the condensation of pyruvate to acetolactate does not occur.

In order to check if any part of the carbon skeleton of glucose is actually used for the synthesis of acetoin, glucose-1-^{14}C, 2-^{14}C and 6-^{14}C were fermented with aliquot parts of a *Leuconostoc* suspension in the presence of a 5-fold excess of pyruvate. After the drop in carbon dioxide production the radioactivity in the acetoin formed was determined. As shown in Table 1, radioactive acetoin is produced only when glucose-6-^{14}C is employed. The specific activity was eight times less than that of the corresponding carbon atoms in the glucose. From this one may conclude that only one-eighth of the synthesized acetoin results from the carbon chain of glucose, the rest from pyruvate. In addition it is seen from Table 1 that only the lower part of glucose can be used for the synthesis of acetoin, and not the C_{2+3} moiety, which appears as alcohol during fermentation. According to the results of Heath *et al.*[5], C_{2+3} first yields acetylphosphate, which is probably reduced further to alcohol via acetaldehyde. Obviously the acetaldehyde cannot be activated and is not used for the synthesis of acetoin. Thus the scheme presented in Fig. 1 may be considered as established for the formation of acetoin in the heterofermentative genus *Leuconostoc*.

In contrast to these findings, Andersen[6] reported recently that *Leuconostoc citrovorum* ATCC 8081 may produce radioactive acetoin from glucose-1-^{14}C as well as from glucose-6-^{14}C, thus behaving like the homofermentative *Str. diacetilactis*. This result may be explained by the observation of Felton and Niven[7] that the mentioned strain ATCC 8081 is not *Leuconostoc citrovorum* but the homofermentative *Pedicoccus cerevisiae*. To demonstrate clearly that our strain represents a heterofermentative *Leuconostoc*, the radioactivity in the fermentation products of spot-labelled glucose was determined. The result obtained is identical with that of Gibbs *et al.*[8]. A more detailed account will be published in *Zentralbl. f. Bakt.*

Table 1. Specific Activity of Acetoin after Fermentation of Labelled Glucose in the Presence of Unlabelled Pyruvate

	Barium carbonate (mgm.)	Specific activity (mμc./m*M* acetoin)
Glucose-1-^{14}C	10·72	0·64
Glucose-2-^{14}C	11·78	0·77
Glucose-6-^{14}C	10·57	32·30

Pyruvate, 500 μ*M*; glucose, 100 μ*M*; manganese chloride, 8 mgm.; calcium chloride, 8 mgm.; phosphate buffer, 0·1 *N*; *p*H 4·8. Total volume, 20 ml.; glucose-1-^{14}C, 89·2 mμc./m*M*; glucose-2-^{14}C, 77·3 mμc./m*M*; glucose-6-^{14}C, 81·0 mμc./m*M*.

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Chloramphenicol, a Simultaneous Carbon and Nitrogen Source for a *Streptomyces* sp. from Egyptian Soil

In work concerning the decomposition of antibiotics in soil and micro-organisms capable of this process, a chloramphenicol solution (250 μgm. chloramphenicol/gm. soil) was percolated through 25-gm. sieved fresh garden soil, *p*H 7·5, in an apparatus similar to that described by Lees and Quastel[1]. The chloramphenicol was applied in three doses; the first dissolved in 50 ml. sterile distilled water and the others in 5 ml. added to the percolate in the reservoir. The antibiotic was bioassayed daily against *Bacillus subtilis*. The first dose disappeared after 11 days and the second and third after 3 days.

Isolation of the micro-organisms involved in the decomposition of chloramphenicol was then carried out by plating the enriched soil in a medium containing the antibiotic as the sole organic carbon source. The medium (ammonium nitrate chloramphenicol medium) contained, in gm. per 100 ml., crystalline magnesium sulphate, 0·5; crystalline ferrous sulphate, 0·003; calcium chloride, 0·005; potassium dihydrogen phosphate, 0·1; dipotassium hydrogen phosphate, 0·25; ammonium nitrate, 0·05; agar, 1·3—adjusted to *p*H 7·0–7·2, dispensed in 100 ml. quantities and autoclaved at 15 lb. for 15 min. Before use, the medium was melted, cooled to 50° C. and chloramphenicol (100 μgm./ml.) added. Plates were incubated at 30° C. for 15 days. Only one type of organism appeared on the plates of the highest dilution (10^{-5}), a *Streptomyces* producing greyish-white thin mycelium and chains of chalky white rod-shaped conidia (1·6 × 0·8μ in stained preparations) borne on long open-spiral conidiophores (section *Spira* in the classification of Pridham, Hesseltine and Benedict[2]). Its cultural characteristics on the media proposed by Waksman[3] were: mycelium greyish-white on Czapek's and starch agar and nutrient gelatin, orange on glucose asparagine, glycerol asparagine and yeast glucose agar media, and lemon-yellow on nutrient agar. Chalky white conidia were produced on all the media except on nutrient gelatin where the growth was markedly poor. Pigmentation of the mycelium appeared to be connected with the heaviness of growth; on media showing non-pigmented mycelia the growth was poorer than that produced on media that revealed pigmentation. Growth in liquid media was restricted to a thin unpigmented pellicle consisting of more or less separate colonies which acquired a chalky white surface when conidia were formed. None of the media tested induced the formation of diffusible pigments. Glucose, saccharose, lactose and mannitol were not utilized. There was no hydrolysis of starch, liquefaction of gelatin, digestion of casein or cellulose, production of ammonia from peptone or reduction of nitrate. Litmus milk turned alkaline after 15 days. Growth took place at 18° and 30° but not at 55° C. The organism resisted pasteurization in glucose broth at 60° C. for 30 min. It had no antagonistic properties when tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Mycobacterium phlei*.

Repeated sub-culture (6 times) in the ammonium nitrate chloramphenicol liquid medium, using straight wire inoculation, always resulted in growth accompanied by the inactivation of the antibiotic, that is, the organism is capable of utilizing chloramphenicol as the sole source of carbon. On sub-culturing in the same manner (6 times) in the liquid medium without ammonium nitrate (chloramphenicol medium) growth and inactivation of the antibiotic again occurred, that is, the organism is capable of utilizing chloramphenicol also as the sole source of nitrogen. This property was confirmed when the growth in the chloramphenicol medium was centrifuged, twice washed with sterile distilled water and used for inoculating fresh media (Table 1). Repeated sub-culturing decreased the inactivation period and improved growth. Inoculation of the liquid medium without chloramphenicol failed to produce visible growth after periods of incubation up to one month.

Table 1. DISAPPEARANCE OF CHLORAMPHENICOL ON REPEATED SUB-CULTURING WITH WASHED *Streptomyces*, INCUBATION AT 30° C.

	100 μgm./ml.*		250 μgm./ml.*	
	Inactivation period (days)	Visible growth†	Inactivation period (days)	Visible growth†
First sub-culture	7	+	20	+
Second ,,	8	++	7	++
Third ,,	3	++	5	++
Fourth ,,	—	—	2	+++

* In non-inoculated controls the variation in diameter of inhibition zones did not exceed ± 0·5 mm. in 20 days incubation.

† Seven days growth on glucose broth ++++.

The ability of the organism to grow in increasing concentrations of chloramphenicol as sole carbon and nitrogen source was tested by inoculating 100 ml. quantities of medium containing 100, 250, 350, 400, 500, 550, 600 and 1,000 μgm. chloramphenicol/ml. with a loopful of 7-day culture in chloramphenicol liquid medium (100 μgm./ml.). Growth of the organism and inactivation of the antibiotic took place in all concentrations up to 600 μgm./ml., where the antibiotic completely disappeared after 13 days. No growth or reduction in activity could be detected within one month in the medium containing 1,000 μgm./ml.

Finally, the purity of the *l*-chloramphenicol used (Kemicetine, Carlo Erba) was verified by the determination of its melting point, carbon, nitrogen, hydrogen and chloride contents and by checking the weight in the capsules supplied.

Work is in progress to elucidate the mechanism of the decomposition of chloramphenicol by the isolate.

We wish to express our thanks to Dr. T. Gibson of the School of Agriculture, Edinburgh, for constructive criticism.

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PSYCHOLOGY

An Unexpected Effect of Attention in Peripheral Vision

THE phenomenon described here suggests an unexpected effect of attention in peripheral vision.

Arrange a number of small objects such as keys, coins, etc., on a table and fixate a point among them from some convenient distance, say 3 ft. Maintaining fixation of the point selected, attend to one of the objects and try to see or describe it in detail.

A number of people including experienced psychologists and physiologists have carried out this procedure and have reported what I had previously found, namely, that the object selected for attention becomes hazy and may even disappear. The effect may persist for seconds at a time or it may be fleeting or fluctuating; but other objects in the field remain steadily visible meantime.

It is well known that the whole visual field may disappear when it is 'stabilized', and some people report that this occurs with steady fixation.

The blurring or disappearance of small regions selected for deliberate attention is, however, a different matter and suggests some form of central interference.

Much remains to be done in determining limiting conditions for the occurrence of the phenomenon and in discovering whether it is affected by training and practice. It is in any event a striking example of attention being associated with a loss rather than a gain in clarity.

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FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, March 6

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Stanislaw Leszczycki (Geographical Institute of the Polish Academy of Sciences): "The Economic Regions of Poland".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. E. D. R. Shearman: "An Investigation on the Usefulness of Back-Scatter Sounding in the Operation of H.F. Broadcasting Services".

SOCIETY OF ENGINEERS (at the GEOLOGICAL SOCIETY, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. H. Kaylor. "Some Aspects of Pre-stressed Concrete".

UNIVERSITY OF LONDON (at Bedford College, Inner Circle, Regent's Park, London, N.W.1), at 5.30 p.m.—Prof. E. Wegmann (Neuchâtel): "Some Aspects of the Geology of the Basements".*

UNIVERSITY OF LONDON (in the Eugenics Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.45 p.m.—Prof. Harald Cramér (Sweden) · "Some Recent Results in the Theory and Applications of Stochastic Processes".* (Further lectures on March 10 and 13.)

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. M. J. Langford: "Photography in Industry and Commerce" (Cantor Lecture).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. Dahlén (Sweden): "Chemical Industry in Sweden".

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Scientific Methods in Archæology". Chairman. Dr. E. T. Hall. Speakers: Dr. C. M. Lerici, Mr. M. J. Aitken and Dr. E. H. Willis.

Monday, March 6—Thursday, March 9

OIL AND COLOUR CHEMISTS' ASSOCIATION (in the Royal Horticultural Society's New Hall, Westminster, London, S.W.1)—Thirteenth Technical Exhibition.*

Tuesday, March 7

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 4 p.m.—Papers on: "Fluidization".

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Prof. Eric Voegelin: "World Empire and the Unity of Mankind" (Stevenson Memorial Lecture).*

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Sir John Macpherson, G.C.M.G. "Sovereign Nigeria".

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. A. V. Hemingway and Mr. R. L. Dressler: : "The Automatic Control of Machines for Assembling Mechanical Components".

UNIVERSITY OF LONDON (at Bedford College, Inner Circle, Regent's Park, London, N.W.1), at 5.30 p.m.—Prof. E. Wegmann (Neuchâtel): "The Folded Chain of the Jura Mountains".*

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, Prince Consort Road, London, S.W.7), at 5.30 p.m.—Dr. C. G. Wynne: "The Correction of Aberration in Optical Systems".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. H. Lundegårdh (Sweden). "Electron Transport Mechanisms in Plant Cells".* (Further lecture on March 8.)

UNIVERSITY OF LONDON (at the School of Pharmacy, 29–39 Brunswick Square, London, W.C.1), at 5.30 p.m.—Prof. H. W. Mossman (University of Wisconsin): "Glandular Tissues of the Mammalian Ovary".*

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. D. T. Swift-Hook: "Magnetohydrodynamic Generation".* (Third of five lectures on "Generation of Electricity without the use of Rotating Machinery".)

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Hatfield Technical College, Roe Green, Hatfield, Herts), at 7.30 p.m.—Mr. D. Hurden: "Some Chemical Problems of Space Flight".

Wednesday, March 8

ZOOLOGICAL SOCIETY OF LONDON (in the Meeting Room of the Zoological Society, Regent's Park, London, N.W.1), at 10.30 a.m.—Symposium on "Aspects of Disease Transmission by Ticks".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINE GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 3 p.m.—Meeting on "The Mechanics of the Formation and Adhesion of Deposits Arising from the Combustion of Liquid Fuel".

PALÆONTOLOGICAL ASSOCIATION (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Annual General Meeting. Prof. T. M. Harris: "The Fossil Cycads" (Fourth Annual Address).

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. Kramer (University of Göttingen): "Renal Blood Flow in Relation to the Counter Current System".*

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Sir Andrew McCance, F.R.S.: "The Steel Industry To-day and To-morrow".

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the CHELSEA COLLEGE OF SCIENCE AND TECHNOLOGY CHEMICAL SOCIETY, at the Chelsea College of Science and Technology, Manresa Road, London, S.W.3), at 7 p.m.—Prof. G. A. D. Haslewood. "Newer Chemistry of Bile Acids and Alcohols".

Thursday, March 9

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. D. P. Craig, Mr. J. M. Hollas, Mr. M. F. Redies and Mr. S. C. Wait: "Analysis of the Naphthalene Vapour Absorption Bands at 3200 Å. I. Naphthalene *h*-8. II. Naphthalene *d*-8".

Friday, March 10

ASSOCIATION OF APPLIED BIOLOGISTS (at Rothamsted Manor, Harpenden, Herts), at 10.50 a.m.—Meeting on "Aspects of Crop Physiology".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. Ronald King. "Energy".

Saturday, March 11

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. C. M. Mitchell: "The Folk Life of the English People".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

BIOCHEMIST for the Wellcome Laboratory, situated at the Veterinary Hospital—The Secretary of the University Court, The University, Glasgow, W.2 (March 10).

JUNIOR RESEARCH FELLOW IN CHEMISTRY—The College Secretary, Balliol College, Oxford (March 11).

LECTURER, and an ASSISTANT LECTURER, IN BOTANY; an ASSISTANT LECTURER IN CHEMISTRY; an ASSISTANT LECTURER IN PHYSIOLOGY; and an ASSISTANT LECTURER IN ZOOLOGY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (March 11).

LECTURER IN APPLIED MECHANICS—The Registrar, The University, Leicester (March 11).

RESEARCH ASSISTANT (BIOLOGIST or CHEMIST) at Poole, Dorset, to assist in research on the tank culture of bivalve shellfish, under the supervision of the professor of zoology, University of Southampton—The Secretary and Registrar, The University, Southampton (March 11).

RESEARCH ASSISTANT (non-medical or medical graduate), to study wound healing by light and electron microscopy—Prof. R. J. Harrison, Anatomy Department, The London Hospital Medical College, Turner Street, London, E.1 (March 11).

ASSISTANT LECTURER IN PHILOSOPHY; and an ASSISTANT LECTURER IN MORAL AND POLITICAL PHILOSOPHY—The Registrar, University College of North Staffordshire, Keele, Staffs (March 14).

RESEARCH ASSISTANT (preferably with experience or an interest in comparative endocrinology or histochemistry) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Nottingham (March 18).

RESEARCH STUDENTS (2) (suitably qualified in zoology, biochemistry or pharmacology) in the Wellcome Laboratories of Pharmacology, the Gatty Marine Laboratory, St. Andrews, for work on thyroid metabolism and physiology of lower vertebrates—Joint Clerk to the University Court, College Gate, St. Andrews (March 18).

LECTURER and an ASSISTANT LECTURER IN PURE MATHEMATICS—Dr. F. G. Friedlander, Secretary of the Appointments Committee of the Faculty of Mathematics, The University, Arts School, Bene't Street, Cambridge (March 22).

ASSISTANT LECTURER (trained in electron microscopic techniques) IN BIOPHYSICS—The Registrar, The University, Manchester 13 (March 25).

LECTURER IN CHEMISTRY (INORGANIC)—The Secretary, The University, Aberdeen (March 25).

LECTURER or ASSISTANT LECTURER (qualified in pure or applied mathematics) IN MATHEMATICS—The Registrar, University College of North Staffordshire, Keele, Staffs (March 25).

PROFESSOR (with a higher degree, preferably a doctorate, and with research experience in the field of animal husbandry) OF ANIMAL HUSBANDRY within the Faculty of Veterinary Science, University of Queensland—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 15).

COMPUTER OFFICER IN THE SUB-DEPARTMENT OF NUMERICAL ANALYSIS—The Registrar, The University, Liverpool, quoting Ref. No. CV/T.

DISTILLERS COMPANY RESEARCH STUDENT IN THE DEPARTMENT OF CHEMICAL ENGINEERING, for work leading to a higher degree on the kinetics of the gaseous oxidation of aromatic hydrocarbons—The Secretary, University College, Gower Street, London, W.C.1.

HEAD OF THE DEPARTMENT OF SCIENCE, Grade II—The Principal, Loughborough College of Further Education, Greenclose Lane, Loughborough.

POST-DOCTORAL FELLOW IN THE CHEMISTRY OF THIOAROMATIC COMPOUNDS IN ACID SOLUTIONS; the research will involve organic chemistry and electron spin resonance spectroscopy—Prof. H. J. Shine, Texas Technological College, Lubbock, Texas, U.S.A.

SCIENTIFIC OFFICER (male or female, with an honours degree in science, pharmacy or biology), for duties consisting of technical work in the Blood Transfusion Service Laboratories—The Director, Blood Transfusion Service, Roby Street, Manchester 1.

SCIENTIFIC OFFICER (with a good honours degree in biochemistry or chemistry and with a particular interest in lipid metabolism), to investigate the interrelationship between sterol and fatty acid metabolism in animals—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, quoting Ref. 61/7.

SENIOR LECTURER (with an honours degree and suitable teaching and research experience, and preferably higher degrees and specialization in modern atomic physics) IN PHYSICS—The Clerk to the Governors, South-West Essex Technical College and School of Art, Forest Road, Walthamstow, London, E.17.

SENIOR TECHNICIAN (preferably with G.C.E. ("A") level in either biology or zoology) IN THE ZOOLOGY DEPARTMENT, to assist in research of Prof. P. B. Medawar and his group on transplantations—The Secretary, University College, Gower Street, London, W.C.1, quoting "Zoology/1".

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

The Species Unit, and Archetypal, Primeval and Primitive Vegetation. By Marietta Pallis. Pp. 20. (Glasgow: Robert Maclehose and Co., Ltd., 1960.) [2411

The Mersey Docks and Harbour Board. Annual Review for the year ended 1st July, 1960. Pp. 16. (Liverpool: Mersey Docks and Harbour Board, 1960.) [2411

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CONSERVATION OF NATURAL RESOURCES

ONE *important gap in responsibility revealed by* the review of scientific effort in Britain made by the Advisory Council on Scientific Policy at the request of the Minister for Science relates to the conservation of natural resources—minerals, land use and biological resources. The responsibilities of the Nature Conservancy cover only limited aspects, and as its annual report shows (see p. 798), even these are interlocked with the responsibilities of other Departments, which may or may not be responsible to the Minister for Science, as well as with the interests and responsibilities of various public or private bodies. The Advisory Council was satisfied that there is a significant absence of basic research in such major problems as the conservation and long-term utilization of water-supplies in Britain, while in formulating the considerations on which policies of land utilization should be based, much remains to be done towards a scientific classification of land capability, including the application of soil science and comprehensive topographical investigations.

On these subjects the Advisory Council is continuing its discussions with the authorities concerned, and meanwhile a study of the competition for rural land by Dr. G. P. Wibberley, of the Department of Agricultural Economics, Wye College, shows the value of research on land reclamation and the need for further effort in this field. The Advisory Council also stressed the need for study of the effect of long-term climatic changes on policy for the conservation of resources, and in view of its suggestion that the application of research in tropical ecology would facilitate the economic planning and execution of development schemes for under-developed countries, it is particularly disappointing to find that the recent report, *Colonial Research 1959–60*, contains no hint that the Overseas Research Council is addressing itself with any vigour to the problem of providing continuity of research in tropical agriculture and medicine in the emergent territories of the Commonwealth. Generally, however, the Advisory Council believes that the conservation of natural resources may be of sufficient importance to justify establishing a research council with central responsibilities, to ensure that, over the whole field, scientific knowledge and research are brought to bear on all these issues; the problem is being considered in detail by the Biology and Allied Sciences Committee of the Council. This suggestion for a further research council found some support in the debate on scientific policy in the House of Lords on November 9, though Lord Shackleton thought that in the first instance the functions and responsibilities of the Nature Conservancy might be extended to enable it to do the work. Lord Hailsham did not specifically reject this suggestion, though he pointed out that the Agricultural Research Council is also working in this field and he thought that we should first have a very clear idea of how any new research council would fit into the general scheme and what sort of work it should be asked to undertake. These matters will doubtless be elucidated by the Advisory Council's sub-committee which is considering the problem; in the meantime the Nature Conservancy states in its annual report that it is already most carefully considering a number of recommendations made by the Advisory Council on subjects of importance to the conservation of natural resources of Britain.

The Nature Conservancy's annual report, however, brings out once again not only inherent inadequacy on the part of the Conservancy to deal with such matters but also the inadequacy of the resources at its disposal. Its grant-in-aid for 1959–60 was no more than £336,000, and after providing some £205,000 for staff and £91,000 for properties and equipment, only some £44,231 was spent on research, training and special surveys, of which £21,509 was in research grants and £14,021 on studentships and training grants. It is scarcely surprising that applications for studentships or for places in the postgraduate diploma course in conservation should far exceed the number of awards the Conservancy is able to make, and the urgent need in conservation for recruits of high calibre to lead and inspire the growing movement of naturalists' trusts continues. Again and again the report refers to fields in which available information is inadequate, but in which no systematic efforts are yet being made to supply the lack; and it is obvious that the directions in which, with its present resources, the Conservancy can increase its efforts are severely limited.

More scientific and technical knowledge about the coast of Britain is needed, and the natural forces and threats affecting it; and on developments here the report quotes as still relevant the judgment two decades ago of the Coastal Preservation Committee, that the question how amenities and facilities shall be designed, where they shall be sited and how the essential characteristics of the coast and the adjoining landscape can be saved from destruction in the process, is urgent and vital in the absence of national control. In this particular field the Conservancy is promoting a fresh survey of the preservation of the coastline of England and Wales, but the report concludes with the judgment: "Almost every day brings new evidence of the price of our present methods of pursuing economic progress. Poisoning of wild life, pollution of seas, shores and rivers, destruction of irreplaceable scientific and amenity features and the exposure of vulnerable flora and fauna to excessive and unreasonable human pressure are among the current threats with which conservation must contend".

Part of the story of the struggle is told in the report, and the Conservancy's hope that enough of the picture will be made plain to awaken public opinion before it is too late should be widely shared by scientists, not least because of the way in which scientific interests themselves have been sacrificed. The picture, however, is not entirely black, and before referring more particularly to the glaring example of Dungeness, some of the encouraging features noted in the present report should be mentioned. First and foremost are the clear signs that the need for conservation, and the efforts of the Conservancy to that end, are becoming better understood and more widely accepted. This is notably true among the universities, as is illustrated by the support given by the University Grants Committee, which enabled the first advanced training course in conservation and ecology to be initiated at University College, London, and by the agreement of the University of Aberdeen to the establishment of the Conservancy's first research unit dealing with grouse and moorland ecology. The Welsh headquarters of the Conservancy and Research Station, opened at Bangor last July, is a notable addition to scientific institutions in Wales; its equipment includes an X-ray vacuum fluorescence spectrograph for elemental analysis of soil and plant material which will greatly reduce the time taken in analysis. An experimental station is also being planned on a site adjoining the Monks' Wood Nature Reserve; this will house the scientific group being formed to study problems of chemical spraying in relation to wild life and also other groups undertaking research on woodland management by natural methods in natural areas, the ecology of vertebrates (particularly mammals and birds) and scientific methods of conservation.

The high esteem for British progress in the field of conservation was manifest when many overseas scientists attended the celebration of the tercentenary of the Royal Society; this event was preceded by a meeting and tour of the British Commonwealth Scientific Committee, in the course of which the heads of all the principal Commonwealth scientific departments visited the Conservancy's Merlewood Research Station, while in June leading workers in this field were entertained and consulted on their way to attend the meetings in Poland of the International Union for the Conservation of Nature and Natural Resources. Evidence of Government interest in conservation was also seen in the promptness with which the Minister of Education acted on a recommendation of the Albemarle Committee and made a grant to the Council for Nature to enable the Conservation Corps to be expanded. The report again pays tribute to the value of the Conservation Corps in easing the difficulties due to the present shortage of field-staff in the reserves.

These and other events have all assisted to create a better-informed public opinion; and the Nature Conservancy has made its own direct efforts to that end. To assist the reconciliation of public access with scientific research and conservation, the Conservancy, in May 1960, signed an agreement with the Hampshire County Council under which the latter undertook to assist, so far as it properly can, towards the preservation of Old Winchester Hill and to help ensure that its extensive use by visitors does not cause injury or disturbance to this fine view-point or to its plants and animals and the studies which are being made of them. The Conservancy has also taken the initiative in seeking compromises or efforts of reconciliation between conservation and other interests in various areas where conservation is threatened by pressure of growing population, and in particular, promising informal discussions have taken place on the preservation of the coast-line.

Reference has already been made to the bearing of the Conservancy's work on wild life and agriculture overseas. The Government's concern for wild life in Africa was explicitly affirmed by the Colonial Secretary in the House of Commons last June, when he said that wild-life conservation measures are continuously under review in the East and Central African territories, but that more use could be made of technical knowledge in developing the wild-life resources, which contribute considerably to local food supplies, as well as being of economic value to the tourist industry. He recognized also the voluntary co-operation of the African populations, and welcomed the prospect of a conference on conservation in Africa being convened in 1961. The deputy-director-general (scientific) of the Conservancy, Dr. E. B. Worthington, has already submitted to the Colonial Secretary a full report on his extensive tour of East and Central Africa during February 7–March 19, 1960, and his report emphasizes the critical importance of conveying to the masses of the indigenous African people the economic importance of the conservation of African wild life and of securing the interest of their leaders. It also indicates the real assistance which the Nature Conservancy can give both in the application of technical knowledge to local management problems and in the training of staff.

Meanwhile, at the meeting of the International Union for the Conservation of Nature and National Resources held in Poland, an African Special Project was accepted as a major activity of the Union during the next three years. This project, which is to be launched in close co-operation with other international agencies, aims particularly at influencing African public opinion towards the conservation and wise use of natural resources, including wild life. Nor, as the report shows, are the overseas functions of the Nature Conservancy limited to Africa or to wild animals.

There are thus wider reasons than domestic ones why every care should be taken that the Conservancy's resources in finance, in staff and equipment are adequate to fulfil the increasing responsibilities which fall on it. Apart from the time factor stressed by the chairman in his report, Britain's scientific prestige is to some extent now involved by the new overseas commitments; and it should be a matter of urgency that the Minister for Science should satisfy himself not merely that adequate resources are available to the Conservancy, but also that the administrative structure is appropriate, including the relations with the Overseas Research Council and the Agricultural Research Council.

Nor is it simply a matter of adequate finance. Government interest must be much more positive than in the past. The Nature Conservancy is dissatisfied, for example, with the protection afforded by Sites of Special Scientific Interest status, and is examining, through discussions with the National Parks Commission, the Council for the Preservation of Rural England and other bodies, the possibilities for improvement. Too often, while sites may be protected against building or other forms of statutory development, the scientific interest is damaged or even obliterated by planting of trees, draining or ploughing, since forestry and agricultural development are not subject to planning control. Moreover, in conservation as a whole, there are still difficulties in assessing with sufficient precision for management purposes the scientific significance of an area.

The gravest threats, however, still come from the Government itself, and the Conservancy's comments on the Dungeness decision make most disturbing reading. In view of the decision announced in July 1959 to authorize a nuclear power station within the boundaries of the proposed reserve at Dungeness, the Conservancy decided not to proceed with the proposed reserve in view of the severe loss to the scientific interest. In announcing this decision on November 24, 1959, the Conservancy said that study of the direct and indirect repercussions of the power station on the remaining area showed that the future scientific interest would be too much reduced to qualify it for the status of a national reserve. The undeveloped area will remain 'designated' as a site of special scientific interest, and the Conservancy will continue to be consulted about any future development which may be proposed for other parts of the area; and in particular it will welcome and encourage any measures which the Royal Society for the Protection of Birds may take for maintaining and defending the fauna and flora, and especially the bird life of the 1,234 acres of shingle which it owns between the power station site, the Lydd Army Ranges and the bungalow settlement.

Nevertheless, as stated at the public inquiry, the issue was that of a clear choice between the conservation of the unique natural interest of Dungeness for scientific research and education, and the exploitation of the area as an engineering site. What is disturbing is that, after scientific interests have been irredeemably sacrificed—and interests which were unique and irreplaceable and could have supplied information of the utmost importance in coastal defence work generally—it should appear that this sacrifice of an important national interest was not really necessary. As the Conservancy's chairman points out in this report, the nuclear power programme has since been revised and it has been decided, for example, that a station on the Wylfa site in Anglesey, approved as suitable, will not be required in the period up to and including 1966.

This possibility was pressed by the Director-General of the Conservancy at the public inquiry at Lydd in December 1958, when he asked if there were not a risk that the nuclear power programme might not be revised again in such a way that the authorization for the site at Dungeness might prove unnecessary. The Chairman of the Conservancy very rightly remarks in the present report that the frequency with which sites of the highest scientific or amenity interest are claimed on grounds of over-riding priority, when experience shows that the reasons given are quite frequently modified even before construction takes place, is a national problem which deserves further attention. The Nature Conservancy recognizes the difficulties of averting such situations; but it does not consider that all possible methods of anticipating them and of minimizing the resultant harm have yet been explored. Its report is submitted to the Minister for Science and refers to a number of matters which call for the Minister's particular attention. None, however, is more important or more urgent than that emphasized in this comment on the Dungeness decision, and it is of over-riding importance that the Minister should take every possible step to ensure that there is no repetition of the Dungeness blunder. If sacrifice of unique and irreplaceable scientific and national interest is demanded, the possibility of doing so in deference to ephemeral requirements should be absolutely excluded. Revision and alternatives should be sought first and not after the sacrifice has been made.

TRANS-ANTARCTIC EXPEDITION 1955—58

Trans-Antarctic Expedition, 1955–1958
Scientific Reports. No. 2: A Gravity Traverse of Antarctica. By J. G. D. Pratt. Pp. 22. 12*s*. 6*d*. net. No. 3: Seismic Soundings across Antarctica. By J. G. D. Pratt. Pp. 69. (London: Trans-Antarctic Expedition Committee, 22 Gayfere Street, S.W.1, 1960.) 30*s*. net.

THE accomplishment of the Trans-Antarctic Expedition as a feat of human endeavour has rightly taken its place alongside the notable journeys of Antarctic history. The first scientific reports by the Expedition's geophysicist, J. G. D. Pratt, have now been published. The work was carried out under very difficult conditions of travel and climate, and early delays during the journey due to crevasses meant that less time was available for scientific work while crossing the plateau than had been anticipated. The amount of work done under these conditions is impressive.

Report No. 2 on the gravity programme deals at length with the performance of the Worden gravimeter. After correcting for the somewhat variable drift of the instrument, the raw gravity results are presented. No calculated gravitational anomalies are given, since the necessary heights above sea-level will not be computed with sufficient accuracy for gravity reduction until appropriate International Geophysical Year weather analyses over the Antarctic continent become available. The author's estimate of possible errors of up to 5 milligals in the raw results seems reasonable. In view of the expected precision of the final elevation measurements, the gravimetric data should be satisfactory for determining the regional anomalies if sufficient measurements of ice thickness are available. Smaller relative errors between adjacent gravity stations should also be satisfactory for giving finer details of sub-glacial relief. A small section on the vertical motion of floating pack-ice and ice shelves, as estimated by the use of the gravimeter, is a useful side-line to the main programme.

Report No. 3 deals with the seismic shooting measurements of ice thickness. The interest of most readers will be centred on the six pages plus one sample seismic recording which deals with reflexion shooting during the crossing of the Antarctic plateau. The difficulties of operating complicated electronic equipment under onerous conditions were clearly overcome. However, the discussion of techniques and the amplitude of the echoes in comparison with the noise-level on the sample record from the South Pole show that much could have been gained had the Expedition been able to take efficient power-drilling equipment on the crossing, so that the seismic shots could have been fired at depths of 20–30 m. or more. This had been shown to be very desirable by similar work on the Antarctic plateau by the Norwegian-British-Swedish Antarctic Expedition, 1949–52. Pratt concludes that the use of greater shot depths is the most promising single line by which improvement of results may be attained, and recommends that equipment to drill to 50 m., if not 100 m., should be carried in future.

The selection for publication of a seismic record obtained at the South Pole could not have been bettered if publication of only one record was practicable. The record is stated to be of typical quality; in due course it is hoped that it can be compared with those of shots fired by two other expeditions at the same point. Pratt marks two weak echoes on his record, the first at an uncorrected arrival time of 1·021 sec., the second at 1·438 sec. The first echo is used to determine the depth of ice at 1,990 m. This compares with a Press report of a depth of ice of about 2,500 m. obtained by Linehan (United States) shortly before Pratt's arrival, and a depth of 2,810 m. obtained by Kapitza (U.S.S.R.) in December, 1959, and published in a paper by Dralkin (*Priroda*, No. 10, 27, 1960). There is no reason for preferring Linehan's preliminary result to Pratt's value, since both were obtained with shot-depths less than 10 m., but since Kapitza fired his shot at a depth of 50 m. one is inclined to prefer his result. If Pratt's second echo (1·438 sec.) is taken to come from the bottom of the ice, it would give a depth in agreement with Kapitza's value. I would agree with the clear discussion of the possibility of errors in picking echoes from the sub-glacial floor, except that Pratt discounts the possibility of noise on the record being mistaken for the required reflexion, as may have happened in this case.

Clearly those interested in measurements of the thickness of ice who are not specialists at seismic shooting will wish to know why different individuals produce different answers for ice depths at one point. On the outer parts of the Antarctic continent, where ice temperatures are above −30° C., it is not difficult to produce first-class results from most areas of inland ice. Some fully reliable results have also been produced on the Antarctic plateau, and there is no doubt that the overall picture of great depths of ice over much of Antarctica is correct. However, under noisy shooting conditions on very cold firn, echoes may not show up clearly against the noise, or a double reflexion may show up when the first reflexion is obscured. Some risk of error clearly arises under these conditions. One can hope to eliminate most errors by making sure that data from seismic soundings, gravimetric measurements, surface slopes of the ice sheet and perhaps magnetic measurements are consistent with one another. The preliminary results of the Norwegian-British-Swedish Antarctic Expedition 1949–52 on the depths of floating ice-shelves had to be reviewed on such a basis. A similar revision of ideas of the thickness of the ice at the U.S.S.R. Vostok station has taken place (Kapitza found a thickness of 3,700 m. compared with an earlier seismic shooting value of 1,800 m.). It is apparent that we shall get the best value from the Trans-Antarctic Expedition results only after a thorough comparison of gravitational anomalies and surface slopes with the possible seismic echoes has been made. It is therefore hoped that when the final report on gravitational anomalies is prepared, such a reassessment of all the data will be made. It will also help the individual research worker if more of the reflexion records obtained while crossing the plateau can be published at the same time

The remaining fifty pages of the report cover work of more specialized interest on refraction shooting at South Ice, and an extensive analysis of the methods and errors involved in determining the thickness of the ice shelf near Shackleton by use of strong reflexions from the sea bottom.

Pratt writes clearly with a rather informal style which shows a healthy disrespect for established ideas that do not have a firm basis. No bibliography is included in either report. The format, printing and general production of the reports are of high quality.

G. DE Q. ROBIN

SAHARAN ETHNOGRAPHY

Tribes of the Sahara

By Dr. Lloyd Cabot Briggs. Pp. xx+295+33 plates. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 32*s*. 6*d*. net.

ACCORDING to Dr. Briggs, "More pure balderdash has been written and repeated about the tribes of the Sahara than about almost any other peoples in the world". In *Tribes of the Sahara* he sets out to correct this, and to show that "the sober truth about the Sahara is more mysterious than anything that has been written about it". But the Sahara is essentially a French literary possession and the mystery and romance which it evokes in French writers disappear when reduced to arid Anglo-Saxon prose. It changes into a mirage of unstable, unattainable hypotheses, and the reader finds himself abandoned in a shifting sea of desiccated gritty facts—that a camel's rate of water elimination is "less than a third of the average figure for mammals in general" (p. 28); that the underground aqueducts locally known as 'foggaras' 'may have been introduced" into the Sahara by the Persians (p. 12); that the best Touareg sword-blades "were imported from Northern

Italy, Germany and Spain" (p. 149); that their kinship structure gives "the distinct impression of a double descent system" (p. 134); that the "sedentary women of Metlili go without trousers as a rule" (p. 208).

The book falls between two stools. It has far too many 'scientific' facts of this sort to make it a successful travel book; on the other hand, there are far too many theories and insufficient supporting data to make it acceptable to anthropologists, archæologists or historians. Human geographers, however, should find it very useful, for it answers their need for a descriptive summary of the peoples of the Central and Western Sahara which is both up to date and accurate, and which takes care to distinguish between fact and fantasy. That the latter still occupies so much space is not a reflexion on Mr. Briggs's scholarship but on the weakness of anthropological fieldwork in this part of Africa. One could have wished, however, that he could have spent less of his twelve years of study in "threshing and winnowing all the Saharan literature that he could lay his hands on", and more time in field research among the Chamba and other representative communities.

The book is supported by two very inadequate sketch maps, thirty-three good plates and an impressive bibliography. G. I. JONES

THE ECOLOGY OF AFRICAN GAME

Wild Life in an African Territory

By Dr. F. Fraser Darling. (A Study made for the Game and Tsetse Control Department of Northern Rhodesia.) Pp. viii+160. (London: Oxford University Press, 1960.) 25*s*. net.

THIS book is primarily a report of a survey carried out by the author in Northern Rhodesia, with the object of examining the present status of the wild African game, the natural vegetation where it survives and the problems involved in the conservation of wild Nature in this territory. Much of the book is thus an account of the journeys undertaken and the terrain examined, followed by an ecological description of the vegetation and the habitats. The book concludes with an account of the measures of nature conservation already undertaken by the Government, and with recommendations for future action. There are, in addition, three appendixes, a directive on wild-life conservation issued by Sir Evelyn Baring as governor of Kenya, an official circular issued to all provincial commissioners in Northern Rhodesia on the same subject, and finally, a paper dealing with the status of the more important ungulates in Northern Rhodesia.

These materials are largely matters of record, though they give an excellent idea of the present condition of the country, and are valuable as a record and for comparison with such earlier data as are available.

In many ways the most interesting part of the book is the relation of this material to the underlying philosophy, and to the reflexions and observations embodied in the introduction and the middle chapters in which the ecology of African biological communities is discussed. Here the author draws freely on his wide experience to generalize. His view is that many of the existing habitats and biological communities in undeveloped Africa represent ecological climaxes which yield the maximum biological production possible under the difficult climatic and ancient soil conditions prevalent. This is mainly because the organisms present include a multitude of highly adapted plants and animals, the diversity of habit and function of which exploits to the full every variety of ecological niche. It follows that a disturbance, and especially a simplification, of the ecological system (such as follow human 'development') is almost certain to lead to degradation of production and to site deterioration. There is therefore a case for maintaining the existing habitats and wild-life systems until further inquiry may disclose methods of land-use which can be fully equated with the natural systems already existing.

Dr. Darling applies this generalization to the particular problem involved in the conservation of the ungulate African animals and their habitats, including the human societies involved in his treatment of this eco-system. He considers that the game can be conserved and also used to give an adequate supply of protein food for Africans in the territory and this, rather than known alternative forms of land-use, offers the best prospect of preserving rather unstable habitats and also perhaps of permitting time for the development of the primitive human social systems. He has succeeded in integrating his material so well as to give a valuable picture of the background and the relation between social life, land-use and ecology in the still savage parts of Africa. Thus the book is an important and useful illustration of the new scientific approach to these problems of wild-life management as well as to the ecology of land-use. W. H. PEARSALL

KINETIC THEORY OF GASES

Elements of the Kinetic Theory of Gases

By Prof. E. A. Guggenheim. (International Encyclopedia of Physical Chemistry and Chemical Physics. Topic 6: The Kinetic Theory of Gases, Vol. 1.) Pp. xii+92. (London and New York: Pergamon Press, 1960.) 17*s*. 6*d*.

THIS is the first part of the *Encyclopedia of Physical Chemistry and Chemical Physics* to be published. It leads us to expect an outstanding work.

Treatments of the kinetic theory of gases have tended to fall into two classes: there are the comprehensive, detailed and somewhat involved accounts which are concerned to be rigorous, regardless of complexity, and, on the other hand, there are simple, approximate accounts, frequently using unsatisfactory assumptions. *Elements of the Kinetic Theory of Gases* seems likely to make a wide appeal to readers who find neither of the two classes satisfactory.

A skilful mathematical formulation of basic kinetic phenomena leads to treatments of successive topics which are both incisive and concise. Clarity is furthered still more by the organization of the material into virtually self-contained, short chapters. The result is that the reader is continually struck by the power of essentially simple procedures which penetrate to the core of a problem, as, for example, in the derivation of Maxwell's distribution law. Again, collision-rates are considered with rigour but without complexity. Simplicity is not shunned even where rigour is lacking, provided it affords insight. The naive treatment of viscosity is given, though to be sure it is followed soon enough by an account which does not assume Maxwellian distribution of velocities; there are parallel treatments of thermal conductivity. On the other hand, no attempt is made to include simple theories in the sections on diffusion and thermal diffusion.

In the final chapters on gas imperfections and intermolecular forces, the fashionable 6–12 potential function is severely scrutinized, and the difficulty of obtaining inter-molecular potential functions from second virial coefficients alone is exposed. A synthetic five-parameter function is advanced and shown, for argon, to give an impressive account of the dependence on temperature not only of the second virial coefficient but also of the entropy, heat content and density of the solid.

The book is aimed at graduate and research workers, but the mathematical treatment is such that undergraduates in their final year could profitably read much of the earlier and later chapters of the book. It is not only part of an encyclopædia to be consulted and referred to, but also a book to be read and enjoyed. D. J. MILLEN

INVISIBLE LUNATIC

Automatic Translation

By D. Yu Panov. Translated from the Russian by R. Kisch. Edited by A. J. Mitchell. Pp. vii+73. (London and New York: Pergamon Press, 1960.) 21*s*. net.

Automatic Language Translation

Lexical and Technical Aspects, with particular reference to Russian. By Prof. Anthony G. Oettinger. (Harvard Monographs in Applied Science, No. 8.) Pp. xix+380. (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1960.) 80*s*. net.

THE title of this review is prompted by the story, until recently apocryphal, that a Russian computer translated the phrase "Out of sight, out of mind" from the English and that an American machine, in due course, re-translated the Russian back into English with the above result.

In so far as this story has a point, it is illustrated by the two books under review. Both are the first generally available technical reports produced by mechanical translation workers in their respective countries. By saying this I do not in any way mean to neglect the volumes of brief essays which have already appeared or the excellent reports, of limited circulation, which some American groups have issued.

Both books describe the activities of single groups of workers, and both are coloured by the particular machines used for the experiments.

Panov's book starts by discussing how human beings go about the act of translation, and then, after examining the characteristics of scientific and technical prose, describes the electronic computer *Besm* 1 and experiments made with its aid in this field. Detailed programmes are analysed and there is a critical survey of the present theories of translation.

Oettinger follows similar lines, except that his machine is the *Univac* 1, and that he is far less concerned with translation theories than with the precise details of Russian–English dictionary construction.

On balance it is fair to say that Panov's book will be far more readable to the non-specialist and, correspondingly, far less useful to workers in the field. Oettinger's book is nearly five times as long and is packed with useful data for the specialist.

Both books are excellently written and can be thoroughly recommended. A. D. BOOTH

ALL ABOUT MANGOES

The Mango

Botany, Cultivation, and Utilization. By Dr. Lal Behari Singh. (World Crops Books.) Pp. xiii+438+54 plates. (London: Leonard Hill (Books), Ltd.; New York: Interscience Publishers, Inc., 1960.) 84*s*. net.

IT is pleasing, as it is unexpected, to find a new series of books on world crops starting off not with such solid fare as wheat or potatoes or cabbage but with something as exotic and appetizing as the mango. This "choicest fruit of Hindoosthan" has spread far beyond the bounds of India and has become one of the most cherished fruits of tropical lands. It is still found in greatest variety and excellence in India, and this book comes, fitly enough, from the pen of a distinguished Indian horticulturist.

The book may be counted on to commend itself as the most complete study of the mango so far published. There has indeed been a recent book, also entitled *The Mango*, which splendidly described the Indian varieties, but had only a short account of their cultivation. Dr. Singh deals with the subject on a broader front, concerning himself not only with the botany and requirements but also with propagation and management, the diseases and pests to be withstood, and the uses to which the mango is put.

While the botanical chapters refer mainly to the common mango, *M. indica*, the author directs attention to fifteen other species of *Mangifera* with edible fruits. Evidently there is great wealth of related material here, of possible value for future breeding. A clear and well-illustrated account is given of methods of vegetative propagation. In describing the practice of cultivation, the author is strangely insistent that the established orchard needs ploughing twice a year to maintain a proper soil tilth. It is not clear why such a disturbance is preferred to maintenance of a more natural leaf-litter. The section on utilization is necessarily brief, since the best way to use a mango is still to eat it as promptly and as dexterously as possible, but some mango recipes are also given. A full bibliography and a good index complete the work.

The book could have done with some tidying up, for misprints and some not quite lucid passages occur. In striving for completeness, the author tends to length, and his more impatient readers may grudge the space given to explaining what a horticultural variety is, or the detail spent on different methods of applying irrigation water to a tree which, over much of its range, is satisfied by the rain from heaven. In giving such full measure, the author doubtless errs on the right side, and his book deserves to be welcomed and to be gratefully added to the small, but growing, collection of hand-books on the tropical crops. T. A. RUSSELL

A TURNING POINT IN THE STUDY OF INSECT MIGRATION*

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C. G. JOHNSON[1] has crystallized a new trend, since the War, in the field of insect migration and dispersal by flight. The trend is inspired largely by work on aphids and locusts, which has been more intensive than that on economically less-important insect migrants such as butterflies. The new knowledge gained cannot be fitted into the previous framework of behavioural ideas due to C. B. Williams[2-4]; therefore Johnson (working on aphids) and Rainey[5] (on locusts) propose shifting the emphasis to the timing and results of migration. This would mean abandoning, along with his particular ideas, Williams's general aim: to characterize migration ecologically, behaviourally and physiologically. The purpose of this article is to suggest that the recent advances, far from giving cause to abandon that aim, have brought it within reach.

Records of migrating insects other than locusts have been collected and collated for many years under the leadership of Williams, and the outstanding achievements of this work are: the demonstration that migration is much commoner than was supposed, even if we count only the long-range and geographically clear-cut cases that Williams considers; and secondly, the detection of apparent return movements in an ever-growing number of species. Lack of evidence of any return in many cases had previously posed a problem of the evolutionary origin of the migratory habit and lent colour to the idea that migration served mainly to reduce high populations[6]. But in all the observations so collated the chief criterion used for distinguishing insects as migrant is the peculiarly persistent, undeviating manner of flight of butterflies, dragonflies, locusts and other large powerful fliers when close to the ground. This gives them a mysteriously purposeful air that rivets the attention of observers and makes teleological interpretation almost irresistible. This in turn proved an impediment to the development of the subject on the physiological and ecological sides alike.

Williams inferred that such migrants continue to fly low, holding to one compass direction for hours and days on end, and in this way directly determine the route of their long-distance movements. This has recently been disproved for locusts and questioned for some butterflies[7], and has not yet been verified for any insect. But taken as fact it posed an intractable problem of the mechanism of orientation. Williams even suggested that migrants had an innate sense of direction independent of the environment. There the matter of direction determination has rested, while other physiological problems of migration have remained in the background.

Furthermore, only insects flying in that self-propelled, self-directed manner were regarded by Williams as migrants. The dispersal of smaller, weaker, more buoyant insects such as winged aphids or moth larvæ that drift on the wind, merely supporting themselves by their wing beating or on silken kites, was not migration. Williams's definitions of migration are to be understood in that exclusive sense: "a change of location which is determined both in distance and direction by the insect itself, and not a passive distribution of individuals by overpowering forces such as strong winds"[3]; or "movement determined by the insect itself; [which] instead of being haphazard in direction, is straightened out so as to result in a change of habitat"[4].

Movements that result in a change of habitat are, of course, of major concern to ecologists[6,8,9]. They, however, regard regular long-range to-and-fro movements between discrete areas as special cases. Hence all the work done on such migration has seemed of doubtful relevance to everyday ecological work. Ecologists use the looser term 'dispersal' for the general phenomenon in which they are interested, and Elton's[8] description of dispersal as "taking place all the time as a result of the normal life of the animals" seems quite the opposite of Williams's basic idea of migration as a distinct biological process. In the absence of more information on dispersal mechanisms, ecologists who do believe there are distinct behavioural 'adaptations for dispersal', have had difficulty in describing them objectively[9].

Recent Advances

Locusts and aphids have, in the past, served as excellent illustrations of the supposed contrast between active migration and passive dispersal. But it turns out that locusts migrate on the wind no less than winged aphids: and winged aphids are, in their own way, no less specialized as migrants than locusts. Both are active, 'deliberate' migrants; and both achieve their long-range movements 'passively'. This is what has made a broadening of the idea of migration seem pressing[1,5].

The new phase opened in the locust work (mainly on the desert locust, *Schistocerca gregaria* Forsk.) when it was realized that the orientation of fliers and the direction of their long-range migrations were separate problems: the one did not directly determine the other[10,11]. The explanation of their purposeful air when seen flying close to the ground does not lie in any type of orienting stimulus capable of maintaining a fixed course and track for long periods. It could be explained in terms of familiar types of orienting stimuli from the immediate environment. These have a steadying effect on courses and tracks near the ground, but certainly do not fix them. For example, low-flying locusts, like butterflies and aphids as well as locust 'hoppers' migrating on foot, change course away from upstanding objects and so keep in the open where locomotion can be maintained. Non-migrating insects frequently turn toward such objects and are arrested.

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* Based on papers contributed by the author to symposia on migration at the eleventh International Congress of Entomology, Vienna, August 18, 1960, and at the Anti-Locust Research Centre, London, September 22, 1960.

But low-level oriented flying achieves relatively little displacement over the ground. The more important way in which the swarming locusts' behaviour ensures that they travel is by their rising actively away from the ground and, moreover, allowing themselves to be lifted by convection, like aphids. In this way, they not only avoid obstacles (including such obstacles as the Atlas Mountains) but also pass out of range of any optomotor orienting effect of the ground pattern and ride the faster-moving upper air. Then, the only obvious orientation reactions are those of locust to locust counteracting the disruptive effects of air turbulence on the swarm. Groups of locusts within it show a common orientation, but this varies from minute to minute and group to group. Cohesion is maintained only because any group that approaches the edge of the swarm, on any side, turns back into it. Meanwhile the swarm as a whole is being bundled across country by the wind[5,11-16].

That this resultantly downwind mode of 'straightened-out' travel can be adaptive was demonstrated by Rainey[16] in an outstanding example of the application of modern synoptic meteorology to insect ecology. The rainfall desert locusts need for breeding occurs in different regions at different seasons, and results from the temporary convergence of winds upon each region, so that the wind-borne swarms always tend to be delivered to the right place at the right time. Thus a more or less regular seasonal to-and-fro pattern of migrations between geographically discrete regions, on the familiar vertebrate model, and by large powerful insects, can be created not by self-directed flights but by 'passive' displacements. It may still be true that the long-range to-and-fro migrations of some butterflies do not depend on such displacement and are actively oriented, but the onus now lies on students of butterflies to prove it.

That the long-range movements of winged aphids are effected by passive transport on the upper air has been very thoroughly confirmed (mainly with the black bean aphid, *Aphis fabae* Scop.)[17,18]. But their embarkment on, remaining in and disembarkment from this transporting vehicle all depend on active locomotor responses[19]. Embarkment depends on a sharp rise in locomotor excitability in the fresh adult which is triggered into flight, and then directed up out of the vegetation into the free-circulating air, by the bright, predominantly short wave-length light from the sky. This oriented 'straightening out' of its movement does not take the aphid far, but again the direct result is a passive, long-range straightening out of its movement by the fast-moving upper air. Disembarkment depends on a change in the reaction to light as flight proceeds, with a weakening of the kinetic effect and of the attraction to sky light and a relative strengthening of the attraction to the longer wave-length light reflected from plants. Before the phototaxis reverses lastingly to negative, there are intermediate phases when the insects fly about actively near the grouud, orienting into and making headway against gentle air-currents and avoiding obstacles, like locusts, and eventually making the alightments by which the flora is sampled for suitable host plants. The intensely active nature of the migratory flight is obvious even in this phase, for the great majority of alighters, even on their own host plants, take off again within minutes.

There is, in fact, a definite inhibition in the young adult of the usual settling, feeding and larvi-position responses to visual and other stimuli from plants, together with an arrest of embryo growth in the ovaries. When the inhibition is eventually removed, by locomotion, embryo growth restarts and at the same time the flight muscles degenerate and the fat body enlarges[19,20]. The restriction of the frenetic migratory activity of these aphids to, at most, a few days and usually a few hours out of the individual's otherwise sedentary life, further underlines the differentiation of migration as a biological function.

What all this means, as Johnson justly insists, is that migration is part of the normal development of winged aphids, "an evolved adaptation"[1]. It is not their reaction to crowding, food shortage or other current adversity as Elton[6], for example, supposed most migrations to be. Nevertheless, although obligate in the winged form of aphid, migration is facultative for the species. Production of the winged form is the response of the wingless form to crowding and other signals of impending adversity. With locusts, too, migration of the persistent lifelong type seen in the gregarious members of swarms is not obligatory but facultative for the species and results from a heritable response to crowding. Migration is often facultative without obvious polymorphism, as in the pierid butterfly *Ascia monuste* L., where it seems to depend on both population density and the time of day of emergence[21]. If, as Southwood argues, migration is usually an adaptation to a temporary habitat[22], then whether it is obligate or facultative may depend on whether the habitat typically lasts long enough for only one generation or for more.

Proposed Non-behavioural Definitions of Migration

Rainey[13] claims with justification that recent work on locusts has "dispelled some of the mystery" in Williams's picture of migration. The mystery was primarily behavioural, and in dispelling it neither Rainey nor Johnson puts anything in its place so far as behaviour is concerned, rather shifting the emphasis to some other aspect of migration. Thus Rainey insists that the effects of vertical and horizontal air-currents on insect displacement must be assessed before the "finer effects" of behaviour, "in order to distinguish what the organism is itself doing from what is being done to it by the environment"[13,16]. But Rainey has himself reported that the members of small swarms do not allow themselves to be dispersed upwards, like aphids, by turbulent convection, when the members of large swarms do[15]. This is but one illustration of the need to assess what both the insect and the environment are doing, simultaneously. Rainey suggests that migration is "more usefully characterized by a seasonal displacement of populations than by directionally purposeful behaviour"[5]. The qualification "seasonal" is not generally applicable since aphids and other insects often migrate in response to irregularly arising local conditions. It is not necessary to fall back on a purely ecological description like "population displacement", for "directionally purposeful behaviour" is no longer the only alternative.

Johnson recommends distinguishing migration flights "chronologically in the first place before attempting to distinguish them behaviourally"[1]. Disregarding migration other than by flight, he adduces evidence that migratory flights usually occur as the first flights of new adults. There are a good many exceptions to this post-teneral rule, notably post-diapause migrations[4]. But most of them, although not all, would be covered if migration in adult insects were characterized as pre-reproductive, rather than post-teneral. This is an important new common feature recognized in migration: a pointer to developmental physiology (below). Nevertheless, it is as new adults that insects first fly anyway, so the timing does not distinguish migration. The timing of an event becomes of interest once we know what the event is.

Johnson gives two reasons for believing there is no satisfactory way of characterizing migration behaviourally[1]. He doubts whether there need be any difference of kind between migratory and other flights, and earlier questioned the validity of Berland's division of the atmosphere into "terrestrial" and "plankton" zones for flying insects[17]. But that general idea has now been revived in Taylor's concept of a "boundary layer"[18]; and Johnson[1] concedes there is a clear difference between migratory and other flights in Lepidoptera and mosquitoes. The difference is equally apparent in locusts[10,23] and aphids[19] and other insects[22]. His second and more important reason for omitting behaviour from his general system is that the behavioural qualities of migrants "are so variable that definitions based on them as to what is or is not 'migration' or what is 'active' migration and what is 'passive' dispersal become arbitrary, controversial and unsatisfactory when applied to all the types of insect that are said to migrate"[1]. It is true that those distinctions have now broken down, because they assumed a single mechanism of "straightening out" of movements, when as we have seen there are two, discussed further below. At the level of reactions to particular stimuli there is great variation; but the resultant straightening out remains a common feature.

Without that straightening out (the "klino-kinetic" component) even very persistent locomotor activity is not migration, for example in a stationary swarm of flying midges. But when combined with it, a second common feature is what Andrewartha and Birch[9] give as the main one: a special readiness to move (the "ortho-kinetic" component). Here again various stimuli and reactions may be involved. Persistent employment of the insect's own locomotor organs is most common; but there may be no more locomotion than that involved in leaving the feeding or hibernation site and releasing the foothold or clambering on the body of a winged migrant[22,24]. But again, a common feature remains in the special readiness to move and go on moving.

Negative Features of Migratory Behaviour

If we consider only those reactions that show heightened excitability (low threshold) in migrants, we cannot make any all-embracing generalization about them except in terms of their resultant effect: straightened-out movement. That common behavioural feature is important; but it does not entirely dispose of Johnson's difficulty, for it does not provide a ready means of distinguishing migration from such long unidirectional movements as those of an ant toward the nest, a chafer toward a clump of trees, a mosquito toward a host, or a male moth toward a female[25]. But a further criterion is available in what the migrant does not do.

In a migrant the persistently lowered threshold of responses resulting in straightened-out movement goes with a persistently raised threshold of responses that arrest and deviate movement. With locusts and aphids in mind, these more-or-less inhibited reflexes have been generalized as "vegetative", for lack of a better term, meaning responses promoting growth, of the larva or of the adult's genital products, through the exploitation of present resources[26]. The locomotor ones among them were called "station-keeping" responses. Migrants are distinguished by "a transient accentuation of locomotor functions with depression of vegetative functions, such that the insect now travels, by what method being a secondary matter"[19]. Nielsen and Nielsen[21], Blunck[27] and Provost[28] directed attention to this unresponsiveness in migrant butterflies and mosquitoes, and Andrewartha and Birch[9] made the same general point. Thus the Nielsens listed the reflex patterns of adult *Ascia monuste* "living in a territory" as feeding, mating, egg-laying, basking and cleaning, and reported failure to respond to the appropriate stimuli for any of these when the butterflies were engaged in unidirectional flight elsewhere.

The relative degree to which the diverse "vegetative" reflexes are inhibited varies greatly. But the grouping of them under one label is no mere descriptive convenience. In the aphid they are allied reflexes in the physiological sense, standing together in antagonistic relationship to locomotion[29]. This is so when the insect is not migrating also. But then the vegetative reflexes are readily excitable and only briefly inhibited centrally by a stimulus that provokes locomotion instead. When migration starts they are deeply and lastingly inhibited. A more complete characterization of migratory behaviour is then: persistent, straightened-out movement that is accompanied by and dependent upon the maintenance of an internal inhibition of those "vegetative" reflexes that will, eventually, arrest movement. Conversely, the locomotor reflexes (or other reflexes securing displacement) cannot for some while be inhibited by stimuli that do promptly inhibit them at other times.

The distance moved is indicative but not always a reliable criterion of migration, as Provost[28] and Andrewartha and Birch[9] point out, because an insect that is ready to respond to an arresting stimulus may not receive it for some time. Hunting and foraging movements may be very extensive, as in some mosquitoes, yet not be migration if the insects stop as soon as feeding stimuli are received. On the other hand, when an army ant colony, on the emergence of a new brood of worker adults, enters on a 'nomadic phase' in which the workers shift the larvæ to a new bivouac site every night along one of the trails they used for foraging that day, this is migration[30]. In the laboratory, starvation produces a high level of locomotor activity in blowflies; but this is not migration physiologically speaking, since the flies take sugar as soon as it is presented and as a result of filling their crops with it become lastingly less active[31]. On the other hand, when locust 'hoppers' or Mormon crickets[32] launch into 'marching' and are stopped only temporarily by suitable food plants, this is migration when compared with the behaviour of the solitary individuals. Young adult dragonflies which disperse in all directions away from the edge of the water in which they developed may also be showing an elementary form of migratory behaviour, because at this time they are positively repelled by the sight of water which will later attract them[33]. Teneral adult *Calliphora* will burrow for hours while they remain in soil, but this is not migration, for emergence from the soil at any time stops the movement[34]. On the other hand, the adult female of *Anopheles sacharovi* may truly migrate when it enters diapause in the autumn, for, unlike the non-diapausing female of previous generations, its flying is apparently not arrested by the first blood meal and shelter it finds, so that it travels far from the breeding place[35].

Thus there is an objective experimental method of deciding whether a moving insect is migrating or not, by measuring its responsiveness to the stimuli signalling the appropriate 'vegetative' requirement. This test has seldom been applied, so that it is often impossible to draw any firm conclusion from the literature. When the insects are known to travel on the upper air the tendency is to assume this is mere 'vagrancy': an 'involuntary' consequence of non-migratory behaviour under special conditions (Williams's "overpowering forces"). The spruce budworm (*Choristoneura fumiferana*) is a good case in point, for it has been studied extensively[24]. Much is known about the immediate triggering stimuli for flight and the air conditions favouring transport, but little about changes in the responsiveness of the individuals. It appears, however, that both larval and adult dispersal are increased actively when the population rises to very damaging densities. The reduced fecundity of the resulting undersized adults is partially compensated by their readiness to fly upwards and thus be transported to undamaged trees before depositing any eggs. The larger, more fecund females produced at less-damaging densities do not fly at all until after they have deposited one or two egg masses[24]. But whether there is any difference of kind between flights before and after oviposition, and whether there is a temporary internal inhibition of oviposition in the undersized females, are still unexplored questions.

The idea of temporary inhibition of 'vegetative' responses in migrants has often been conveyed in a subjective, teleological way that confuses means with ends and serves as a substitute for, rather than an invitation to, experiment. Migratory behaviour has been distinguished as not searching (for any vegetative requirement: food, mate, oviposition site, etc.), not internally oriented, non-motivated, non-appetitive[9,21,28]. The inference is that any movement with a specifiable end is not migratory (see Provost[28] on pre-hibernation flights of anophelines). But migratory and non-migratory movements both end with readiness to respond to some specific stimulus signalling a vegetative requirement or resting place, and in neither case is the movement directed by its end. In both, the locomotion is, in fact, centrally antagonistic to the reflexes that terminate it, at first inhibiting them and finally being inhibited by them in turn. The only objective sense in which a movement is directed toward an end is that the antagonistic arresting reflexes may be progressively 'primed' (thresholds lowered) as a result of being inhibited by the locomotion (successive induction[29]). The basic difference between migratory and non-migratory behaviour is a difference of relative thresholds. There is no sharp line of demarcation between them; we are concerned rather with degrees of functional differentiation in a particular direction, and our first concern is to identify that direction.

Physiological Differentiation in Migrants

The study of insect migration will remain in its present backward state until we know more of the sensory, nervous, endocrine and metabolic components of the mechanism by which migration is 'primed' (in addition to the immediate stimuli that elicit the primed responses) and likewise of the reverse changes that follow on migratory activity. The best information of this kind concerns aphids[30]; but generally there is little. Something is known about fat deposition in place of egg development in migrants[4] as in diapausing insects, about flight muscle development in place of eggs[36] and the use of fat as the most economical flight fuel for migrants[37].

Ecologically, migration sometimes looks like an alternative to diapause when conditions will no longer permit growth, development or reproduction[9,22]. It is so when the habitat deterioration is merely local so that vegetative activities can be resumed earlier elsewhere than *in situ*. It is not so when the deterioration is synchronized throughout the insect's range, as in a temperate autumn or dry tropical summer. Migration may then be, on the contrary, a condition for diapause, if that is also not possible *in situ*[4,22]. But physiologically, the important point is that migration is in either case at the expense of vegetative functions in the individual. The internal inhibition of certain reflexes is the behavioural component of a whole physiological syndrome.

Fig. 1 illustrates this by comparing the timing and amount of growth or reproduction in migratory and non-migratory (or less migratory) females of the same species in the laboratory, where actual migratory activity is minimal and vegetative requirements are continuously available. Solitary locusts (Fig. 1, right) migrate as young adults between seasonal habitats, but the syndrome is developed to an extreme in gregarious locusts which migrate throughout their lives and will not settle in any place[33,26]. Gregarious locusts may show hastened instead of retarded oviposition when there is a tendency to diapause governed by length of day, but in either case they lay considerably fewer eggs[41,42]. The garden chafer (*Phyllopertha borticola* (L.)) is another interesting variant[43]. All the females are ready to lay on emergence before any flight, and most lay their eggs then and do not migrate. The migrants are females that did not lay the bulk of their eggs on emergence and thereafter seem to have developed an inhibition of the reflexes associated with laying; they lay readily after migrating.

The nature of the physiological syndrome in migrants—accentuation of 'sensori-motor' functions with depression of vegetative ones—makes it easier

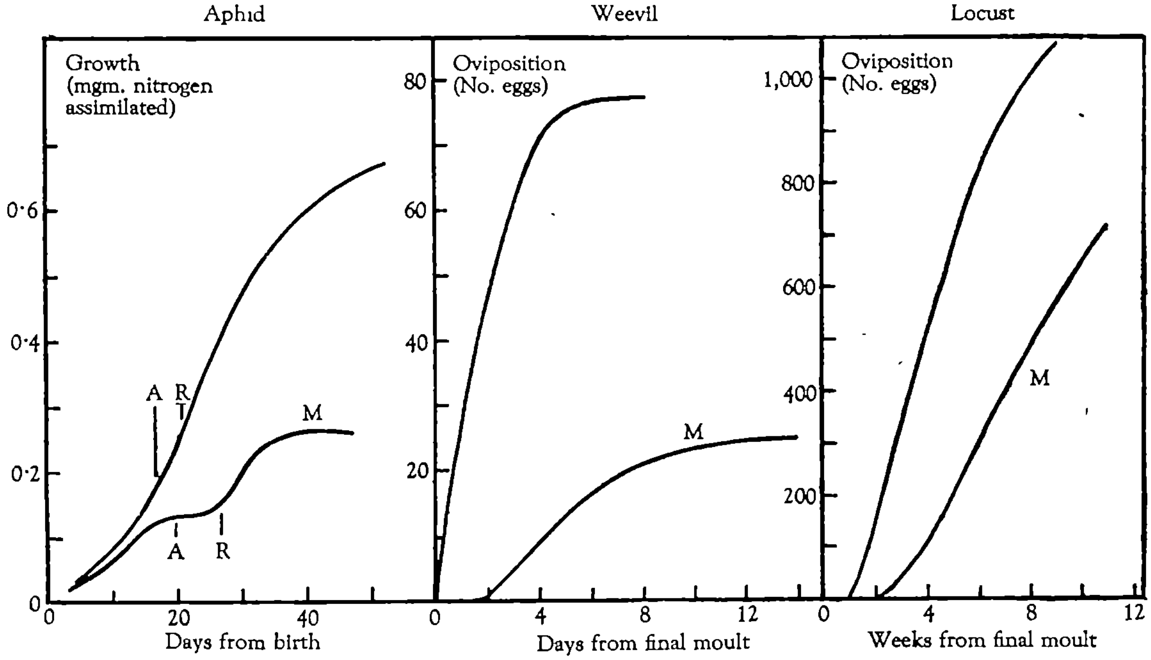

Fig. 1. Examples of the arrest and depression of growth functions in adult migrants (*M* curves). Left: *Tuberolachnus salignus* winged (*M*) compared with wingless form, after Mittler (ref. 38). *A*, final moult to adult; *R*, start of reproduction (larvi-position) after which the further rise represents embryo growth; Centre: *Callosobruchus maculatus* 'active' (*M*) compared with normal form (terminology of Caswell (ref. 39)), after Utida (ref. 40); Right: *Locusta migratoria migratorioides* gregarious (*M*) compared with solitary form, after Norris (ref. 41). All curves cumulative

to understand why migration so commonly occurs in the adult stage and is then usually pre-reproductive[1] or inter-reproductive. For reproduction involves a resumption of the growth functions typical of juveniles, and "an individual is at its most adult during that period of physiological reversal wherein the products of one growth phase are not added to but employed and consumed in ensuring the success of the next"[36]. In this sense migration is physiologically 'super-adult'. In that light it is equally understandable that the next most common stage at which migration differentiates is the first instar larva. This stage is better fitted than later larval instars, because it often has to travel farther than them at any rate when the eggs are not laid directly on the food, it is smaller and hence more buoyant, and more numerous.

At the same time the negative features of migration represent a differentiation in the direction of diapause. Diapause cannot be characterized by one physiological process in all insects. The one common feature is temporary failure to respond by further growth or development to the conditions that will, eventually, promote those processes[44]. Considered physiologically, migration is an example of this rather than an alternative to it.

During the depression of vegetative functions in both migration and diapause some other process occurs which is a pre-condition for their resumption. In diapause this unidentified other process is called "diapause development"[44]. In the winged aphid the equivalent of diapause development is promoted by an antagonistic process, locomotion, which restarts the arrested vegetative functions by central nervous induction[29]. This distinguishes a *locomotory* type of developmental arrest from other types, such as arrests of larval development that are broken by feeding, arrests of egg development or oviposition broken by copulation, and diapauses proper in which the nature of the response promoting diapause development is still unknown, but the stimuli are known to be low temperature or photoperiod[44]. By no means all the developmental arrests in migrants can be assumed to resemble the aphid's, for some are typical extended diapauses covering also periods of complete inactivity[4]. But in the common situation where the developmental arrest ends with the travelling, it is suggested as a working hypothesis that the two are causally related as in the aphid.

It follows from all this that when, as frequently happens, a deterioration in individual size, fecundity or other vegetative properties is observed under certain conditions, this should not be taken as wholly negative in effect until the possibility of an accompanying increase in migratory capacity has been excluded.

Ecological Functions of Straightened-out Movement

Resultantly downwind displacement independently of orientation was not recognized as a migratory "straightening-out" mechanism by Williams. But it also brings about long-distance travel, instead of confinement within a small area through frequent changes of direction, and does so on a larger scale than does oriented movement on or near the ground; and it results from the active, specialized behaviour of the insects. In locusts, aphids and many other insects[1,32] this consists in rising actively away from the ground and so out of what Taylor[18] calls the "boundary layer" within which non-migrants actively confine their movements. Such resultantly downwind travel cannot therefore be dismissed as the "passive distribution of individuals by overpowering forces", which Williams[2] excluded from migration. It can be assimilated readily into his definitions of migration cited in the first section above, provided we do not read into them his restrictions.

Moreover, these two mechanisms of straightening out, actively oriented and passively wind-borne, can be observed at different times in the same migrant, powerful locust and weak aphid alike. Migrants cannot therefore be separated into two classes according to which mechanism they exhibit, although they may differ in the relative importance of the two mechanisms in their migration. After their oriented ascent, aphids use the wind-borne mechanism in the earlier part and the low oriented one toward the end of a migration[19]. Garden chafer migrants seem to use the two more equally from the start[43]. According to the available accounts[2-4,21], many butterfly migrants keep close to the ground more persistently than aphids, chafers or locusts, and may thus use the oriented mechanism during a greater proportion of their time, although this does not necessarily mean for the greater part of the distance they travel.

Hitherto, it has been solely in the context of seasonal to-and-fro migrations between geographically discrete regions that the ecological function of straightening out seems to have been considered. Williams's hypothesis that straightening out is directionally adaptive, serving to direct the migrants to the right place at the right time, provided the motive for his successful search for evidence of return movements; and his hypothesis has now been confirmed for the seasonal movements of locusts by Rainey[16]. But the continued migratory movements of locust swarms through the mosaic of soils and vegetation within a wind convergence zone where suitable food plants and oviposition sites occur in patches are not directionally adaptive. Some long-range seasonal movements of aphids and other small plant-feeding insects seem to deliver them also to suitable breeding areas[44]. But again this is not true of their movements within very considerable regions wherein the host plant is not concentrated in one place but patchily distributed. Many sorts of habitat, hibernation site, etc., are similarly scattered.

In that situation migrants are no more likely to find what they require in one direction than in any other, or at one distance more than another. The straightening out of their movements has then no directionally adaptive significance. They travel in many directions by both straightening-out mechanisms; and this is adaptive inasmuch as some of them find the requirements in all directions and at all distances. Given enough individuals, the two mechanisms together make an efficient 'scanning' device that works on a coarse and a fine scale by means of long wind-borne, and short, low-level, oriented traverses, respectively.

Straightening out here serves the simpler and, presumably, more primitive function of displacement *per se*: the opposite of 'station keeping'. This is not, of course, an efficient procedure so far as the individual is concerned. The positive advantage of having large numbers of individuals if the migratory habit is to develop[19,26] is a point that has been little considered in discussions of the association between density and migration. Enormous losses of individuals *en route* certainly occur, and even entirely abortive movements by enormous numbers of individuals together, as in Elton's famous case of the lachnids on Spitsbergen. But these losses the species can evidently afford; and if migration tends to be associated with temporary habitats[32] this may mean that such habitats tend to permit higher multiplication-rates than permanent ones. The losses do not call in question the adaptive value of the behaviour pattern that brings them about because the same behaviour brings about biologically successful movements as well, with the added advantages of escape from overcrowding, enemies. etc. What the procedure does achieve is a very efficient coverage of the available habitats over a large area and avoidance of 'too many eggs in one basket'[19].

Given multi-directional movements between places arranged in mosaic the evolutionary problem of return movements disappears, as Wiltshire[26] pointed out in connexion with butterfly migrations. Many species of aphid alternate seasonally between woody and herbaceous plants. It has never been supposed that the descendants of a given individual necessarily returned to the same tree the same year. Nevertheless, untidy, constantly reshuffled returns are assumed, and no problem has been raised of how the migratory habit could persist in the population. The same applies to any insects that repeatedly disperse and colonize new habitats available at the time, new or old, so that no particular movement can be singled out as a return.

Movements of this kind are so common as to be of most interest to ecologists, but are included under dispersal rather than migration[9] because they are not directionally adaptive. Since that is a secondary matter it seems best to apply the term migration, as Johnson[1] and Southwood[32] are now in effect doing, to all cases where a change of location can be shown to be an active 'evolved adaptation' along the lines distinguished here, regardless of exactly how or at what stage in the life-history it occurs. The continued use of dispersal instead of migration for the particular mechanism of transport by wind or water can cause only confusion. Dispersal will, no doubt, continue to be used in the purely spatial sense of population scattering, especially when a loose term is needed because there is not yet enough information to judge whether the movement is migratory or not. If it is used in any more precise sense this should be specified. Thus dispersal is likely to be used for non-migratory movements involving no general inhibition of vegetative reflexes, but merely a change in them, as when the adult's food is different from the larva's; or for movements that may involve some temporary inhibition of them, as when new-hatched caterpillars scatter over a uniformly palatable leaf, but are not persistent or straightened out enough to rate as migration.

To sum up: recent work has undermined C. B. Williams's behavioural assumptions about insect migration while confirming his idea that it is a differentiated biological process. Migration is a persistent, straightened-out movement with some internal inhibition of the responses that will eventually arrest it. It may be effected by the insect's own locomotory exertions or by its active embarkment on some transporting vehicle. These are the behavioural aspects of a physiological syndrome which is 'super-adult' in character but may develop at any stage of the life-history, and in common with diapause involves a phase of depression of 'vegetative' (growth-promoting) functions as a condition for their resumption.

Grateful acknowledgment is made to Drs. A. D. Lees, P. E. Madge, T. R. E. Southwood and B. P. Uvarov, and to Prof. V. B. Wigglesworth, for their criticisms of this article in draft.

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FLUCTUATIONS IN EQUATORIAL STRATOSPHERIC WINDS

By R. A. EBDON and R. G. VERYARD

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THE predominance and persistence of easterly winds, especially above the level of the tropopause, is a well-known feature of the airflow over equatorial regions. This could be attributed to an inflow of air towards the equator; air moving towards the equator tends to retain the angular momentum about the Earth's axis which it possesses as a result of the Earth's rotation, and it therefore tends to arrive at the equator as an easterly wind. But westerly winds, that is, air moving faster than the equator, can also be produced over equatorial regions if there is a pressure gradient along the equator. However, as the net pressure gradient around the equator must be zero, a predominance of westerly winds is not to be expected; indeed, a westerly airflow encircling the equator is very difficult to explain. That there are, in fact, westerly air-streams at certain levels over some equatorial regions has been known for some time.

There are, for example, the large-scale westerlies in the lower troposphere associated with the flow patterns of the monsoons; but these are not surprising because of the substantial pressure gradients in the monsoon regions. But an explanation in terms of the pressure pattern of the occurrence of quasi-permanent westerlies in the equatorial stratosphere, sometimes called the 'Berson' westerlies (because it was A. Berson who first directed attention to the existence at about 20 km. of a narrow belt of westerly winds in the lower stratosphere over central Africa), has not proved practicable.

Moreover, it has been alleged by some workers that the Berson westerlies constitute a narrow ribbon of west winds extending right round the equator, and, as indicated above, this presents a challenge to dynamical meteorology. However, the idea which has been given in many meteorological publications, that the equatorial stratospheric westerlies occur with a regularity and persistence comparable to that of the equatorial tropospheric easterlies, needs some modification. Indeed, it would appear that in the lower equatorial stratosphere the zonal component of the wind, that is, both the easterlies and the westerlies, is subject to marked fluctuations—one regime replacing the other at intervals of a year or more[1-3]. Not only do data (based on radar wind ascents reaching 50 mb. and above) indicate that, for some months, there can be very considerable variations in the equatorial stratospheric winds between one year and the next, but they also show that westerly winds can occur not merely in a shallow layer but also throughout the

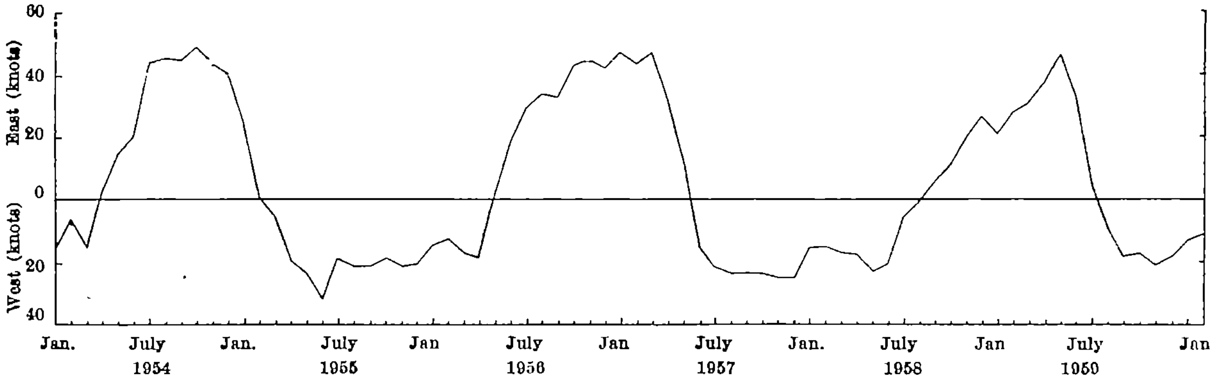

Fig. 1. Monthly mean values of 50-mb zonal wind component at Canton Island

lower stratosphere from the tropopause upwards to above 30 mb.

The monthly mean values of the zonal wind components at 50 mb. for Canton Island (02° 46′ S., 171° 43′ W.) for the period January 1954–February 1960 are shown in Fig. 1. It will be seen that there is a fluctuation with a period of about 25–27 months. Although only a few years data are available for analysis, this fluctuation is also evident in Fig. 2, which gives a curve of the monthly mean values of the zonal wind component at 60 mb. at Nairobi (01° 18′ S., 36° 45′ E.) and in Fig. 3 giving similar curves at 30, 40, 50 and 60 mb. for Christmas Island (02° 00′ N., 157° 23′ W.). At these stratospheric levels the wind direction at the stations considered is nearly always either almost due easterly or almost due westerly, except during the months when the change-over from one regime to another occurs. Once a predominantly easterly or westerly flow is established it tends to persist for some 10–13 months, although in any one month there may be a few days when the direction is reversed. The in-phase agreement between the 60-mb. curves for Nairobi and Christmas Island is quite remarkable. Similar results have been obtained for the zonal winds at 60,000 ft. (about 65 mb.) for the period 1954–60 at Singapore (01° 20′ N., 103° 53′ E.); and shorter period data for the 50-mb. level at Recife (08° S. 35° 53′ W.) show that the phenomenon also occurs at that station. Indeed, the evidence suggests that the fluctuation occurs more or less simultaneously at the same level all round the equator. In any event, the results indicate that, in the equatorial belt, a mean monthly (or yearly) wind obtained by averaging over a period of years will be very misleading if regarded as representative of the average wind in any particular month (or year), as is commonly done in climatology.

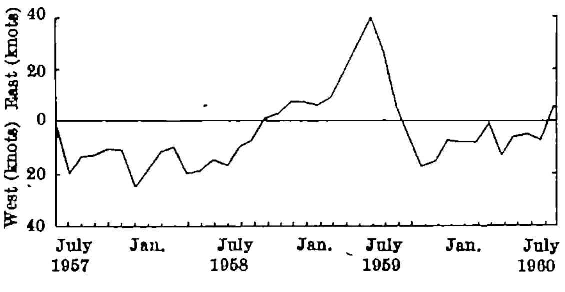

Fig. 2. Monthly mean values of 60-mb. zonal wind component at Nairobi

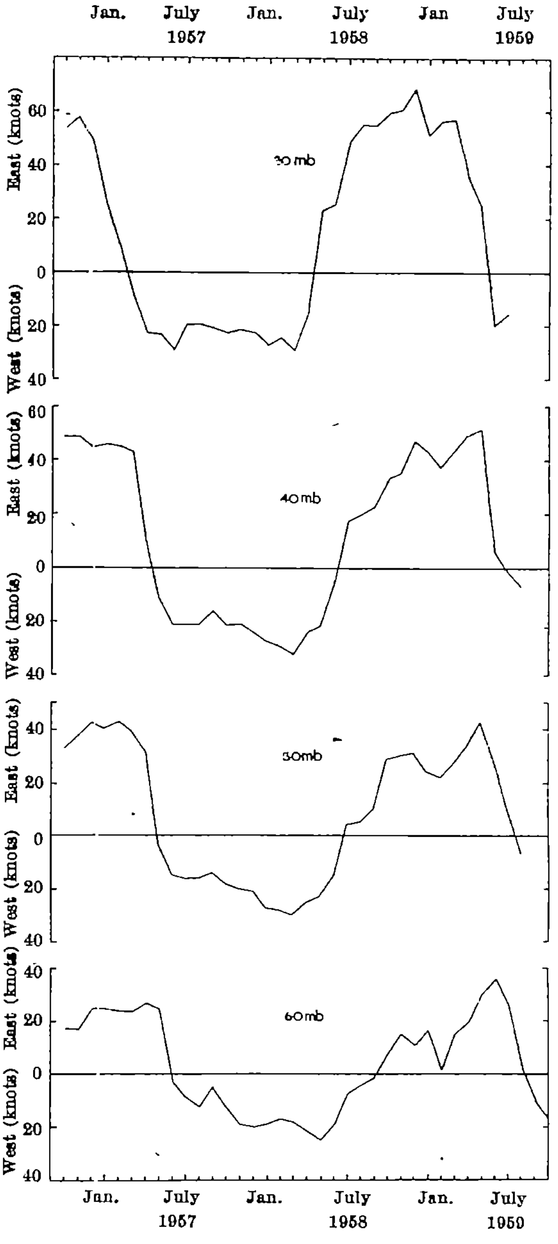

Fig. 3. Monthly mean values of zonal wind component at Christmas Island at 30 mb., 40 mb., 50 mb. and 60 mb.

Table 1. MONTHS DURING WHICH THE MEAN ZONAL WIND COMPONENT AT CHRISTMAS ISLAND CHANGED SIGN

Level	From east to west	From west to east	From east to west
10 mb.		(Oct. 1957)–(Nov. 1957)	
20 mb.	Oct. 1956–Nov. 1956	(Dec. 1957)–(Jan. 1958)	
25 mb.	Dec. 1956–Jan. 1957	Mar. 1958–Apr. 1958	Apr. 1959–May 1959
30 mb.	Feb. 1957–Mar. 1957	Apr. 1958–May 1958	May 1959–June 1959
40 mb.	Apr. 1957–May 1957	June 1958–July 1958	June 1959–July 1959
50 mb.	Apr. 1957–May 1957	June 1958–July 1958	July 1959–Aug. 1959
60 mb.	May 1957–June 1957	Sept. 1958–Oct. 1958	Aug. 1959–Sept. 1959

Table 1 shows the months during which the mean zonal wind component at levels above 60 mb. at Christmas Island changed from easterly to westerly or vice versa. (The two bracketed entries are based on the maximum winds reported, as insufficient data were available to give monthly mean values.) It will be seen that, as was found by Viezee[2], the change in wind direction occurs first in the higher stratosphere and then gradually descends. The one occasion when it was possible to detect the change at 10 mb. suggests that it takes almost a year before the change in wind direction at 10 mb. reaches 60 mb. When the change in wind direction occurs at 25 mb. it is some 6 months later before the corresponding change occurs at 60 mb. The curves in Fig. 2 show that the amplitude of the fluctuation decreases downwards.

From such investigations as have been possible with the data available there was no detectable connexion between these events in the equatorial stratosphere and events in the equatorial troposphere. If adequate data are available, it is hoped to determine the extent to which the fluctuation extends north and south of the equator. Results so far obtained suggest that within 10° the fluctuation still exists but with decreased amplitude.

This communication is published by permission of the Director General of the Meteorological Office, and the subject is discussed in detail in a paper which will be published elsewhere.

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NEWS and VIEWS

Mineralogy and Petrology at Cambridge :

Prof. C. E. Tilley, F.R.S.

PROF. C. E. TILLEY is retiring from the professorship of mineralogy and petrology in the University of Cambridge at the end of the present academical year. On the retirement of Prof. Hutchinson and of Dr. Harker in 1931, the professorship of mineralogy and the readership in petrology were combined in a new chair of mineralogy and petrology, of which Prof. Tilley was the first holder. During his tenure of thirty years he has not only been exceptionally active in research, but also has been the leader in building a department of world-wide influence in its field. In addition to teaching and research in mineralogy and petrology, the Department has contributed much to the teaching of crystallography and to the crystallographic study of minerals. Prof. Tilley has played a full part in university life, having served on the Board of Research Studies, the General Board and the Council of the Senate. He was also for many years chairman of the Faculty Board of Physics and Chemistry, and was vice-master of Emmanuel College from 1952 until 1958.

Prof. W. A. Deer

PROF. W. A. DEER, who has been appointed to succeed Prof. Tilley, has held the chair of geology in the University of Manchester for eleven years. He has brought into existence there a thriving research school, the distinctive feature of which is the application of modern physico-chemical techniques to the solution of geological problems. In 1953, together with Prof. L. R. Wager, of the University of Oxford, he led a geological expedition to East Greenland, continuing investigations which had been interrupted by the Second World War. He has since directed various mineralogical researches on the rocks of the Cape Edvard Holm area, most of them still unpublished, but all of the high standard that has always characterized his work. Of recent years, he has spent much time writing a new book on rock-forming minerals in association with two members of the Manchester staff, Dr. R. A. Howie and Dr. J. Zussman. Active as he has been in geological work, Prof. Deer has found time to play an ever-increasing part in general university affairs. He has been chairman of the Executive Committee of the General Board of Faculties, and of the Science and Technology Section, and has served on committees too numerous to mention here. Particular reference should, however, be made to his work in the implementation of the extensive building programme on which the University of Manchester is now engaged.

British Drop Forging Research Association :

Mr. P. H. R. Lane

MR. P. H. R. LANE, head of the Members' Service Department of the British Welding Research Association, has been appointed the first director of research of the Drop Forging Research Association. Mr. Lane was educated at Alleyn's School, Dulwich, and Strand School, Elm Park. He obtained an honours degree in metallurgy at the University of London and afterwards joined the staff of the British Welding Research Association. He was first engaged in metallurgical work on the weldability of low-alloy high-tensile steels. In 1950, he transferred to the engineering section to work on the design of pressure vessels and pipe-line components, and for five years was in charge of the department dealing with this work. During this period he was also associated with work on the brittle fracture of steel plates and on the fatigue strength of welded components. In 1957, he returned to metallurgical work as deputy chief metallurgist of the Association, in charge of all ferrous metallurgical investigations. In 1959, he was appointed to the new position of head of the Members' Service Department, which is responsible for all contacts with the members of the Association. Mr. Lane is an associate of the Institution of Metallurgists, and an associate member of the Institute of Welding. He has published many papers on both the metallurgical and engineering aspects of welded fabrications and also on the techniques of experimental stress analysis.

The Mellon Institute: Research Directors

Drs. A. A. Bothner-By, Thomas G. Fox and Harold P. Klug have been appointed research directors in the Mellon Institute, Pittsburgh. Dr. Bothner-By joined the Institute in 1958 as a Staff Fellow and has been an assistant director of research since 1959. He is recognized as an authority on the use of nuclear magnetic resonance in organic chemistry, and has developed a centre of research in the latter field at the Institute. Dr. Fox joined the Mellon Institute in 1957 as an assistant director of research and has headed a large part of the Institute's extensive polymer research programme. He is known for his work in the chemistry and physics of rubber and other polymers. Dr. Klug joined the Institute in 1945 as an Administrative Fellow after eighteen years as a professor of chemistry in various universities. He became an assistant director of research in 1959. A specialist in structural physical chemistry, Dr. Klug is the author of two books and numerous papers.

Cultural Contacts in the British Commonwealth

Replying to questions in the House of Commons on March 2, Mr. D. Sandys, Secretary of State for Commonwealth Relations, said that the Government wishes to encourage the development of closer links between young people in the Commonwealth, and, besides initiating a programme for providing 5,000 additional places for overseas students in hostels where they will live and mix with British students, it proposes also to provide a number of new or enlarged social and cultural centres to be run by the British Council. The Government is prepared to meet the capital expenditure involved up to a maximum of £3 million, and will, as at present, consider applications for financial assistance to voluntary bodies concerned with Commonwealth youth. It is hoped, however, that private support will also be forthcoming for such projects and that the voluntary organizations active in this field would bear some share of the cost. Mr. Sandys expressed the Government's appreciation of the work of Field Marshal Sir Gerald Templer's Committee on this question. He said that the Government would welcome any reciprocal action in this sense among Commonwealth countries, and that the British Council had been authorized to consult voluntary organizations, the universities and other educational authorities and institutions about the whole programme. He undertook to see that the nature of the scheme was made widely known throughout the Commonwealth.

The Dounreay Fast Reactor

In written replies to questions in the House of Commons on February 28 about the Dounreay fast reactor, the Parliamentary Secretary for Science, Mr. D. Freeth, said that the work at Dounreay, though widely diversified, is concerned mainly with developing the fast reactor system. Construction of the fast reactor was completed during 1959 and a series of low-power tests have been completed; tests in the megawatt range will shortly begin. The reactor will then be gradually worked up to full power. The reactor first went critical in November 1959, and the main problems encountered since have not been concerned with the nuclear side of the experiment. No major change in the level of work at Dounreay is contemplated. Mr. Freeth also said that he hoped to make a statement shortly about a low-power nuclear reactor for the universities and colleges of technology.

Scientific and Technical Personnel in American Industry

A survey of the employment of scientific and technical personnel in private industry in the United States in January 1959 was conducted for the National Science Foundation by the Bureau of Labour Statistics of the Department of Labour as part of a comprehensive programme of studies of scientific and technical personnel in all sectors of the economy (Pp. vi + 66. Washington, D.C.: Government Printing Office, 1960. 45 cents). Estimates showed that of 800,000 scientists and engineers employed in American industry in January 1959. 764,000 were employed by the 47,500 firms covered by the survey. Engineers numbered 615,000 or 80 per cent, and the 149,000 scientists included 72,000 chemists, 18,000 biologists, 15,000 physicists and 15,000 geo-scientists. Aircraft, electrical equipment, chemicals and machinery accounted for 45 per cent of the scientists and engineers covered by the survey; but in the industries covered scientists and engineers represented only about 3 per cent of the employees, varying from 11 per cent in aircraft manufacturing to 0·3 per cent in textiles and apparel. In manufacturing industries, firms with 5,000 or more employees accounted for nearly two-thirds of the scientists and engineers, but only two-fifths of the total labour force. Of the 277,000 scientists and engineers employed in research and development. about 5 per cent were employed in administration; 235,000 were employed full-time in research and development, the remaining 40,000 being engaged also to a significant extent in other activities. Only in production and operational work were more scientists and engineers employed than in research and development, inspection quality control, or other production and operational work accounting for 39 per cent of the total. Almost 550,000 technicians, or about 72 for every hundred scientists and engineers, were also employed, and of these about 250,000 were classified as engineering and physical science technicians, more than 195,000 as draughtsmen, and 16,000 as medical, agricultural and biological technicians. In research and development about 156,000 technicians were employed, or 56 per 100 scientists or engineers. A comparison with previous surveys indicates that the employment of scientists and engineers grew much less in the years immediately prior to 1959 than in 1954–57, and even in research and development the rate of increase decreased from 13 per cent per annum between 1954 and 1957 to 7 per cent between 1957 and 1959; the increase being largest with mathematicians (17 per cent) and smallest for chemists (3 per cent).

Research in Biochemistry and Allied Sciences in Great Britain

At the Cardiff meeting of the British Association for the Advancement of Science in September 1960, a discussion was held, under the joint auspices of the Biochemical Society and Section I (Physiology and Biochemistry) of the Association, to consider the financing of biochemical research in Britain (see *Nature*, 188, 194; 1960). Because of their wide interest, the Biochemical Society has now published the proceedings of this meeting (*The Organization and Financing of Research in Biochemistry and Allied Sciences in Great Britain.* Edited by K. S. Dodgson. Pp. 51. London: The Biochemical Society, 1960. 2*s*. 6*d*.). Nine speakers were chosen, who were particularly concerned with the organizing and financing of biochemical research in Britain. After an introduction by Prof. A. Haddow, the role of the following organizations was described: Medical Research Council (Dr. F. J. C. Herrald), Agricultural Research Council (Sir William Slater), Department of Scientific and Industrial Research (Mr. C. Jolliffe), and the Imperial Cancer Research Fund (Dr. G. F. Marrian). Prof. F. C. Happold discussed problems of a university department of biochemistry, Dr. F. A. Robinson dealt with biochemical research in industry, and Dr. J. B. Bateman discussed American support of biochemical research in Great Britain. Prof. A. Neuberger summarized the proceedings of the meeting.

The Commonwealth Forestry Bureau, Oxford

THE work of the Commonwealth Forestry Bureau at Oxford has been described and published as an example of the work done by the Commonwealth Agricultural Bureaux, of which it is the youngest member (*A Synopsis of the Work and Growth of the Commonwealth Forestry Bureau, Oxford, 1939–1959.* Pp. 27. Oxford: Commonwealth Forestry Bureau, 1960. 2*s*. 6*d*.). The Bureau was formed in 1939 to serve foresters, wood technologists and scientists in associated fields throughout the Commonwealth, but from the first the quarterly publication, *Forestry Abstracts*, attracted subscriptions from foreign countries, and the Bureau and its periodical are now internationally known; the abstracts reach more than ninety countries. The principal work of the Bureau is the quarterly publication of *Forestry Abstracts*, which notices, by abstract or by title only, more than 5,000 publications annually on forestry and associated subjects.

Medicine and Public Health in China

IN a paper presented at the meeting of the American Association for the Advancement of Science on December 27, Dr. William Y. Chen, U.S. Public Health Service, discussed the condition of medicine and public health in modern China. The main sources of information for the preparation of the paper were various medical and public health journals published both in Chinese and English during the past ten years. The picture is presented against the background of medicine and public health in pre-Communist China, and shows that the most remarkable accomplishment has been the prevention and control of many infectious and parasitic diseases which have ravaged the country for generations. There has also been an improvement in medical education in quantity if not in quality. Other progress in medicine does not seem to correspond with the claims made for it.

Problems in Cybernetics

A VALUABLE new addition to the steadily growing list of translated Russian scientific journals has recently appeared; it is called *Problems in Cybernetics* (Pergamon Press. Pp. x+314. Russian editor, A. A. Lyapunov), and it is to be published at irregular intervals. The first issue contains a general article by Lyapunov, entitled "Certain General Problems in Cybernetics", which serves to show just what fields of inquiry the Russians include under the term 'cybernetics' at present (for the boundaries are very nebulous). Particular emphasis seems to be placed on automatic computer programming, for the simulation of all types of control process, including those analogous to certain biological processes; on representation of information and its processing, including that in automatic programme-controlled computers; on mechanization of various thought-like processes, as in decision making, recognition, strategy and various 'goal-seeking' operations. It is planned that the journal will contain, in the future, articles dealing with the theory of algorithms and automatic devices, theory of control and problems of production automation, theory of operations and of calculating machines, and with certain applications of the ideas of cybernetics to biology, economics and linguistics. Also, material will be included of a critical, bibliographical and historical nature, being primarily details of the work of the cybernetics seminar held in the University of Moscow. Particular reference is made to this seminar, which has been held since 1955, with a list of titles of papers; these include work on conditioned reflexes primarily. There are also listed titles of papers read at a technical conference held in 1957 which suggest wider fields of interest; especially applications to linguistics, to automatic translation, to coding of chemical formulæ, to speech analysis, to machine-reading, together with many technological aspects of computer design.

The Association of Universities of the British Commonwealth

THE report of the Executive Council of the Association of Universities of the British Commonwealth for the year ended July 31, 1960, records a membership of 103 universities, 17 university colleges and 4 other approved institutions of higher learning (Pp. 30. London: Association of Universities of the British Commonwealth, 1960). The volume of appointments work continued to increase, and, in one way or another, the Association helped with 877 vacant posts and dealt, mainly by correspondence, with 4,782 inquiries; 1,514 applications were received and the universities announced the appointment of 139 of the candidates who applied through the Association; of these 72 were in Australia, 28 in New Zealand and 20 in Hong Kong. Some account is given of the Association's work under the Commonwealth Scholarship and Fellowship Plan, and of its administration of the Frank Knox Memorial fellowships, the Marshall scholarships, the Imperial Relations Trust scholarships and of the Commonwealth University Interchange, as, for example, in connexion with the new scholarship scheme of the Drapers' Company to enable British schoolboys to take their first degrees at certain overseas Commonwealth universities. Some account of the work of the Committee of Vice-Chancellors and Principals of the Universities of the United Kingdom is appended, together with the accounts of the Association, and a note on the financial statement which records total expenditure of £48,089 and income of £52,187.

The Transvaal Museum

At the present time, museums are extending their ancillary services in many directions while still maintaining their essential function of the collection and preservation of objects. It is interesting to note in this connexion that the Transvaal Museum is sponsoring a bat banding project to serve as an aid in the study of these animals (*Bulletin Transvaal Museum*, No. 5. Pp. 8. July 1960). At present the emphasis is on cave bats and species of the genera *Rhinolophus*, *Miniopterus* and *Myotis* are being dealt with, though house bats such as Tadaridae have also been banded. While most of the work is at present concentrated on the Transvaal, banding in Bechuanaland and Natal has also been carried out. Bats have been recaptured over a maximum period of nearly two years and over the longest distance of about 100 miles. Chameleons have been kept in the museum for the purpose of studying behaviour and the flap-necked, dwarf, forest and horned chameleons have been included in this research. Particular attention has been given to a comparison of the aspects of behaviour in related species and genera.

National Museum of Wales

The fifty-third annual report of the National Museum of Wales for the year ended August 1960 records the welcome grant to the Welsh Folk Museum of £10,000 towards the cost of constructing a new car park and ticket office and for widening the approach road to the new entrance (Pp. 78+4 plates. Cardiff: National Museum of Wales, 1960). In the Department of Geology the systematic and stratigraphical fossil collections have been re-housed, and the model of the Penrhyn Slate quarry and collection of miners' lamps transferred to the new Industry Gallery. The artist in the Department of Botany has made models of 23 flowering plants, all of which replace the less attractive dried material previously on show. In the Glanely Gallery, Department of Zoology, the exhibit of birds' nests and eggs has been reorganized and augmented, and casts have been used to replace spirit specimens. In the Department of Archæology notable progress has been made in modernizing the Roman Gallery, and a careful watch has been maintained for chance discoveries. The Department of Industry was officially opened in May 1960, which event naturally culminated a year of preparation. The Department of Art has been much engaged in the work associated with temporary exhibitions. In this short note it is only possible to mention the highlights in the work of this progressive and stimulating museum.

Nomenclature of Human Chromosomes

The rapid growth of knowledge of human chromosomes and their importance in genetics and medicine have led to some confusion about nomenclature in the literature. Consequently a small study group of workers recently met in Denver, Colorado, to formulate an acceptable scheme of nomenclature (*J. Hered.*, 51, 214; 1960). It was agreed to number the chromosomes serially, from 1 to 22, as nearly as possible in descending order of length; that the sex chromosomes should continue to be referred to as X and Y, and that the 22 autosomes should be classified into 7 groups, distinction between which can readily be made. Within these groups, especially that comprising chromosomes 6 to 12, and including also the X-chromosome, identification of the chromosomes is difficult by present criteria, though it is believed that with very favourable preparations all or most of the chromosomes can be identified. Two valuable suggestions emerged from the discussions. One concerned the storage of documentation for reference, perhaps in a central repository; the other referred to the need to preserve cultures for future reference, comparison and exchange.

Studentships and Fellowships in Agriculture

The Agricultural Research Council has issued a useful booklet giving particulars of the thirty postgraduate studentships offered in 1961, tenable for one to three years, and including a limited number of veterinary training grants, of four years duration, given to enable science graduates to read for a veterinary qualification (Pp. 20. London: Agricultural Research Council, 1961). The studentships, which are open to honours graduates in science or those holding a veterinary qualification, or to graduates in agriculture or horticulture who have shown special interests in one of the basic sciences, and veterinary training grants vary from £340 to £520 per annum tax free, together with educational fees. Particulars are also given of three agricultural research fellowships of £700 to £1,000 per annum, tenable for up to three years, offered to honours graduates of not less than three years postgraduate experience, and two veterinary research fellowships of £900, rising to £1,000 per annum, tenable for up to three years, open to qualified veterinary workers with not less than three years experience in veterinary research. The booklet also describes the scope of agricultural research and outlines the openings available to agricultural research and advisory work.

Biographical Material relating to Richard Owen

Prof. Jacob W. Gruber, associate professor of anthropology, Temple University, Philadelphia, Penn., U.S.A., writes: "During the past few months, I have been preparing a catalogue of the collection of Owen correspondence in the possession of the British Museum (Natural History). It has occurred to me that it would be valuable to bring together as many materials relating to the life of Richard Owen as may still be extant. I would, therefore, appreciate any information relating to the nature and whereabouts of such materials".

The Indian Society of Genetics and Plant Breeding

The following have been elected officers and councillors of the Indian Society of Genetics and Plant Breeding: *President*, Dr. E. K. Janaki Ammal; *Vice-presidents*, Dr. A. R. Gopal-Ayengar, Dr. A. B. Joshi; *Secretary*, Dr. M. S. Swaminathan; *Editor*, Dr. B. P. Pal; *Councillors*, Dr. S. M. Sikka, Dr. S. Govindaswamy, Prof. P. N. Bhaduri, Dr. B. S. Kadam, Shri G. P. Argikar, Dr. K. Ramiah.

Royal Society Visiting Professorship

The Council of the Royal Society has appointed Prof. R. Couteaux, professor of animal biology, University of Paris, to be Royal Society visiting professor for the academic year 1961–62. He is expected to take up the appointment on October 1 and work in the Department of Biophysics at University College, London, on the structure and function of neuro-muscular connexions. Prof. Couteaux is a distinguished histologist who has made outstanding contributions by microscopic and histochemical means to our knowledge of synaptic structures.

University News: Birmingham

The title of first assistant to the chair of pathology has been conferred on Dr. D. B. Brewer, reader in pathology. The following appointments have been made: Dr. M. Z. Atassi, to be Research Fellow in the Department of Chemistry; Dr. D. A. Smith, to be lecturer in the Department of Microbiology; M. J. Hamlin, to be lecturer in the Department of Civil Engineering; Dr. B. Cockayne, to be lecturer in the Department of Physical Metallurgy.

The thirty-first annual report of the University of Birmingham Research Committee covers the session 1959–60 (Pp. 137+1 plate. Birmingham: The University, 1961). Besides summarizing research progress in the various departments during the session, it gives, arranged by departments under faculties, particulars of work published during the year and titles of theses approved for higher degrees. There is also a selected list of general works and writings by members of the University staff, not included in the departmental research bibliographies; this is arranged by faculty.

Cardiff

It has been announced that Dr. H. K. M. Lloyd, at present senior lecturer in metallurgy at the University of Nottingham, has been appointed to succeed Prof. W. R. D. Jones in the chair of metallurgy at the University College of South Wales and Monmouthshire, Cardiff.

London

Dr. R. A. Shooter, reader in bacteriology at St. Bartholomew's Hospital Medical College, has been appointed to the chair of bacteriology tenable at that College. Mr. L. H. Hamlyn, lecturer at University College, has been appointed to the University readership in histology tenable at that College. The title of reader in physics in the University of London has been conferred on Dr. R. O. Davies in respect of his post at Queen Mary College.

Manchester

Prof. B. H. Flowers, who has held the chair of theoretical physics since 1958, has been granted the title of Langworthy professor of physics, and will continue to be responsible for the teaching and research in theoretical physics. Two other chairs in physics, one vacated by Prof. Samuel Devons and one recently authorized, have been filled by the appointments of Dr. E. B. Paul (from a date to be arranged) and of Dr. H. E. Hall (from March 1). The following appointments have also been announced: Dr. J. Hoenig, to be reader in psychiatry; Dr. M. F. Lappert, to be senior lecturer in chemistry. Dr. J. F. Adams has been appointed a reader in mathematics from a date to be arranged.

Announcements

Prof. C. H. Townes, professor of physics at Columbia University, has been awarded the Rumford Premium of the American Academy of Arts and Sciences for his work on microwave amplification by stimulated emission of radiation (maser). The award consists of two medals, one of gold and the other of silver, and 5,000 dollars.

Lord Nelson of Stafford has been elected an honorary member of the Institution of Electrical Engineers in recognition of his contributions to the development of electrical science and engineering, and for his services to the Institution.

Julius A. Stratton, president of the Massachusetts Institute of Technology, has been awarded the Faraday Medal of the Institution of Electrical Engineers for his contributions in the fields of technological education and research in radio communication.

Mr. P. Lambert has been appointed head of the Engineering Section of the British Steel Castings Research Association, Sheffield. Mr. Lambert joined the Association's staff in September 1955, as a senior investigator in the Plant Engineering Section, paying special attention to problems arising in fettling shop operations. During the past year he has been engaged on liaison work with member companies relating to the simplification of work.

The annual conference of the Institute of Metal Finishing (incorporating the Electrodepositors' Technical Society) is to be held at Llandudno during May 2–6. Further information can be obtained from the Conference Secretary, Institute of Metal Finishing, 32 Great Ormond Street, London, W.C.1.

A meteorological field course is being organized jointly by the Royal Meteorological Society and the Field Studies Council, and is to be held at Preston Montford during April 5–12. The course is particularly designed for sixth-form grammar school pupils. Further information can be obtained from the Assistant Secretary, Royal Meteorological Society, 49 Cromwell Road, London, S.W.7.

The spring conference of the X-ray Analysis Group of the Institute of Physics and the Physical Society, entitled "The Crystal Structures of Organic Compounds", will be held in the Chemistry Department, University of Glasgow, during April 6–7. Further information can be obtained from the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

An international symposium on "Criticality Control in Chemical and Metallurgical Plant", organized by the Organization for European Economic Cooperation's European Nuclear Energy Agency in collaboration with Kernreaktor Bau- und Betriebsgesellschaft m.b.H., is to be held during May 2–5 at the Karlsruhe Nuclear Research Centre in Germany. Further information can be obtained from O.E.E.C. European Nuclear Energy Agency, 38 Boulevard Suchet, Paris 16e.

The fifth European Congress on Molecular Spectroscopy is to be held in Amsterdam during May 29–June 3. The Congress is being sponsored by the International Union of Pure and Applied Chemistry, the Koninklijke Nederlandse Chemische Vereniging and the Nederlandse Natuurkundige Vereniging. Further information can be obtained from Dr. D. H. Zijp, Anorganisch Chemisch Laboratorium, Nieuwe Achtergracht 123, Amsterdam-C, Netherlands.

Erratum. The recent announcement concerning Dr. D. C. Rose in these pages (*Nature*, 189, 444; 1961) is incorrect; Dr. Rose has in fact been appointed associate director of the Division of Pure Physics of the National Research Council, Canada.

THE NATURE CONSERVANCY

ANNUAL REPORT

THE eleventh annual report of the Nature Conservancy, covering the year ended September 30, 1960*, records a number of encouraging features to which reference is made on p. 779. Planning and initiating scientific management on the national reserves continued to be a major activity and the six new management plans approved by the Conservancy during the year brought the total of reserves managed under plans to 39, or nearly half the total declared. In Scotland 16 out of 24 declared reserves are now managed under approved plans; in England 19 out of 42; and in Wales 4 out of 17. High priority was given to increasing the cadre of field-staff such as warden naturalists, wardens and estate workers, but outside assistance is still needed. Another major task of the conservation staff is concerned with the sites of special scientific interest, and in England and Wales the revision of county schedules, timed to coincide with quinquennial revisions of county development plans, is now well advanced. Four new national Nature reserves were established during the year: 2 in Scotland, 1 in Wales and 1 in England. With 4 extensions, the 84 reserves at the end of September covered 138,991 acres.

During the year, 10 new grants for research, totalling £7,173, were approved, and 7 existing grants were extended or renewed, bringing the expenditure on grants to some £16,529 compared with £10,217 a year ago. Fourteen studentships were awarded, 20 were continued and 6 applications for third-year extensions were approved. Lists of these research grants and studentships, with lists of reserves, publications and of the Conservancy's committees are appended to the report. With special grants from the University Grants Committee, a one-year postgraduate diploma course in conservancy was set up at University College, London, and 6 additional studentship awards were made to enable the 6 students accepted to attend this course.

Scientific research again covered a wide field. At Merlewood Research Station, the major research continued to be on woodlands and particularly to elucidate as fully as possible the biological and physical processes in which the soils and living organisms of a woodland are involved, more especially the amounts of nutrients for plant-growth available in the soil, the rate at which they are taken up, and the processes by which the supply is maintained. An important part of the work in this field was carried out in three sets of forest plots, at West Tofts in the Breckland, Abbotswood in the Forest of Dean and at Bedgebury in Kent; and from the observations obtained it was possible to draw up a major part of the balance sheet for nutrients in the plots and to assess the productivity of the different tree species. These investigations show that losses of nutrients from the ecosystems of forest plots which are incurred by the felling and extraction of the stems are not so great as for agricultural crops, the most serious potential losses being those of phosphorus and calcium. Some badly needed information on the rate of supply of minerals by soil weathering and the rate of loss by leaching may in the future be acquired from the experimental plots at Gisburn Forest, set up jointly by the Forestry Commission and the Nature Conservancy. The present work of the Plant Soil Section is concentrated on the study of the nutrient cycles in irregular woodlands of semi-natural structure, and along with studies on breakdown of litter an extensive survey has been made of woodland soil animals. To facilitate comparisons of the nutrient cycles and productivities of natural and managed woodlands, an attempt is being made to provide samples of woodland resembling what is believed to be the natural state.

At Moor House, besides continued work on the productivity of grasslands and the growth of tree species in upland areas, a broad survey of the plant ecology of such areas was commenced and a geological survey, including a map of the reserve and an outline investigation of the peat stratigraphy and vegetation history, as shown by pollen analysis, is almost ready for publication. Investigations on the mountain grasslands and soils of Snowdonia also continued, and in the study of soils attention has been given to the distribution of iron within them, while work continued on the trace element content (particularly of cobalt and copper) of soils of direct interest to the research programme as a whole. Differential grazing experiments at Cwm Idwal and on the south-east slopes of Snowdon show that, with complete protection from grazing, *Calluna vulgaris*, *Vaccinium myrtillus* and *Erica tetralix* are increasing on the more acidic soils, and taller-growing grasses, such as *Deschampsia caespitosa* on the more basic sites, though, in such instances, some of the former groups of species are gaining a foothold. A detailed study has commenced of the connexion between the considerable differences in climate and vegetation within Scotland, and the work of re-establishing and encouraging the regeneration of woodlands on nature reserves in Scotland continued to expand. The latest evidence arising out of the work of recording of chalk grassland transects in southern England is that, despite control measures, rabbits have returned in such numbers on a few sites that their grazing is reversing the changes in vegetation.

Work on the ant population of east Dorset heathland at Furzebrook indicates that the effect of fire has been to destroy their population directly and indirectly in various ways, or that they are now spread more thinly over a greater area. Investigation of the food and population cycle of the short-tailed vole (*Microtus agrestis hirtus*) at the Carron Valley will be completed during the present year. A new Unit of Grouse and Moorland Ecology was set up in co-operation with the University of Aberdeen. The pilot red grouse inquiry financed by the Scottish Landowners' Federation indicated that grouse numbers are usually controlled by the birds themselves, and the main problem before the Unit is to discover how the limits to numbers are set. Ecological research on British reptiles continued at the Furzebrook Research Station, and the programmes of marking grey seal (*Halichoerus grypus*) pups on the Farne Islands, the Pembrokeshire coast and in the Hebrides have been more closely co-ordinated. An analysis of all results to date undertaken in consultation with the Zoological Society of London showed that the maximum period between the marking and the recovery of a seal is no more than 61 weeks.

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* Report of the Nature Conservancy for the year ended 30th September, 1960. Pp. vii+138+14 plates. (London: H.M. Stationery Office, 1960.) 6s. 6d. net.

In coastal research, experimental studies at Orfordness demonstrated that the movement of beach material on any coast is not necessarily uni-directoral. The work at Newborough Warren, Anglesey, has now established that the continued mobility of the dunes produces a cyclic interchange between the marram grass community of the dunes and the creeping willow community of the slacks, which could have a period of about 80 years in parts of the dune system at Newborough, although the cycle may be interrupted or extended at any stage by associated changes in the surrounding dunes. The importance of climatological research is also stressed. The Climatological Section at Aviemore provides observations and advice for other research sections and for conservation and regional officers concerned with managing the reserves; and the Moor House Reserve is of exceptional interest as being possibly on the very threshold of climatic conditions suitable for blanket fog development and maintenance, and this and similar thresholds are to form a chief line of research in the Climatological Section.

As usual, the work of the Scientific Advisory Services has considerable scientific interest. In hydrology, the Conservancy has some small-scale experiments in progress which can be expected to contribute useful preliminary information on the effects of land management; but there is real need for field experiments on different catchment areas on a larger scale and by a specialized research unit. Formal representations were made to the Minister for Science regarding the risks to wild-life from toxic chemicals used in agriculture, and a Unit has been established to investigate the subject. The Conservancy also views with great concern the increasing disregard by highway authorities of Circular 718 relating to chemical spraying of roadside verges, and has made representations to the Ministry of Transport. The Conservancy also emphasizes the urgent need for continued forethought and action on the part of the fire service, railways, agriculture, the Nature Conservancy itself and tourism and others to initiate research into the causes of fires in forests, heaths and moors, and into the appliances and techniques for their prevention and control. Investigations into the heavy mortality among foxes suggests that seed dressing with dieldrin and similar chemicals may be a cause. An investigation is planned into the food of red deer, and the Conservancy has also stressed the need for a full scientific appraisal of the status of the coypu (*Myocaster coypus*) in Britain.

SELF-REGULATION IN LIVING SYSTEMS

A SYMPOSIUM on "Self-Regulation in Living Systems", organized by Dr. D. K. C. MacDonald, was held at the National Research Council in Ottawa on January 6. More than thirty people took part, and, as at previous meetings (*Nature*, 184, 239 (1959); 183, 370 (1959); 187, 298 (1960)), these represented many different scientific disciplines, including engineering, neurology, philosophy, physics and physiology. The five papers presented all provoked lively discussion.

In the morning Dr. Bernice Grafstein (McGill University, Montreal) first dealt with the reception of peripheral information, that is, how information is carried from the surface of the body to the central nervous system. By way of introduction, Dr. Grafstein described the structure of a sensory neurone, consisting essentially of a sensory nerve ending which receives the external impulse and a long fibre or 'axon' which transmits the signal to the central nervous system. The transmission takes place with finite velocity by means of progressive displacement of electrical charge through the membrane of the axon. This charge displacement involves essentially a positive feedback mechanism and the resulting 'action potential' is consequently an 'all-or-none' event. A neural message consists of a train of action potential pulses, the coding mechanism being the spacing between the pulses. There are different types of sensory endings sensitive to different stimuli, and there is an orderly spatial arrangement of terminations in the cerebral cortex corresponding to different parts of the body. Thus the central nervous system is informed about the nature and location of the stimulus by which nerves are stimulated. The main part of Dr. Grafstein's talk was concerned with the modulation of the pulse-train in relation to the strength and time-variation of the stimulus. Examples of records of action potentials were shown to illustrate that usually application of an external stimulus gives an initial rapid burst of activity followed by a slower steady activity. For many types of receptor it appears that the initial frequency of action potential activity is dependent on the rate of application of the stimulus, whereas the steady final level depends (often logarithmically) on the magnitude of the stimulus.

Dr. Grafstein considered in greater detail the case of the muscle spindle as a sense receptor. By inserting an electrode very close to the spindle (rather than merely measuring the action potential at a distance down the axon) one can measure a local steady potential at the receptor, the so-called generator potential. On application of the stimulus, this generator potential goes through an initial peak to a final maintained level, the initial peak being related to the rate of stretch of the muscle, and the maintained potential depending on the degree of stretch. There is a fair linear correlation between the final frequency of the action potential pulses and the final generator potential-level. Precisely how the stimulus is converted into a generator potential in the receptor is at present unknown. The generator potential signal is passed down the axon as a digitally coded (or perhaps better pulse-frequency modulated ?)

train of action potentials, and it appears that the message is integrated and reconverted into an amplitude-coded form in the central nervous system.

Dr. H. K. Hartline (Rockefeller Institute) then described measurements of the action potential in optical nerve fibres. In this case the receptor in the eye contains a protein substance which undergoes a reversible photochemical reaction on absorption of light. A generator potential is then produced (by an undiscovered mechanism) and the message is transmitted to the brain by action potentials in the optic pathway similar to those described above. In this case, the final rate of activity (after the initial burst) is correlated logarithmically with the brightness of incident light. Dr. Hartline's main thesis was to emphasize that data-processing starts right at the receptor and is not confined to the brain. The receptor passes on to the brain only information 'it considers relevant', and, to quote Dr. Hartline, this often involves a terrible distortion of truth, as it accentuates sudden changes occurring in time and space. Dr. Hartline described experiments on the horseshoe crab, the eye of which is a simple structure made up of a number of separate facets or elements. Electrodes are connected to one particular element of the eye so that the output of a single receptor can be measured. It is found that the output from nearby facets are not independent; for example, the response from a particular element decreases when a nearby element is illuminated. There is a linear relation between the decrease of frequency of one facet and the activity above a certain threshold value of another facet closer than a few millimetres. Dr. Hartline had some lantern demonstrations remarkable in their simplicity to show that this same 'distortion of truth' occurs in the human eye also; for example, a wedge the brightness of which as measured by a densitometer varies uniformly between a light and a dark background appears to have a light band just inside the light background and a dark band within the dark background. This to some extent gives the lie to the old adage that 'seeing is believing'.

In the afternoon Dr. Bradford Dunham (International Business Machines) discussed the computer itself as an object of scientific curiosity. He mentioned three ways of studying computers. The first is by formal studies as initiated by Turing thirty years ago; a Turing machine is a conceptual computer obeying a specific set of simple rules, and the object of the exercise is then to see how much the computer can accomplish. Turing's method appears of limited value; in particular, it is difficult to correlate formal studies with practical conditions. The second method of approach is to construct or simulate a 'baby' machine, a small computer with limited range, and to understand as fully as possible its mode of operation in its own little restricted world. The results are again often doubtful in application, as it is usually by no means certain whether particular conclusions are merely a specific consequence of smallness or whether they are indeed of wider validity. The third way of studying the potentialities of computers is to observe how a computer deals with a particular class of problems. Dr. Dunham described experiments made at International Business Machines to programme a computer to perform automatic design, amplifying what he had said at an earlier symposium (*Nature*, 184, 239; 1959). He pointed out analogies between the method followed by the machine and the nineteenth-century Darwinian theory of design by natural selection in many generations. Dr. Dunham remarked that the method has been used to design parts for a rocket mechanism, and a long discussion ensued.

The following paper was presented by Dr. Rolf Landauer (International Business Machines) on the necessity for friction in decision-making systems. Thermal agitation in a system has two related effects: friction or dissipation of energy, and random impulses or noise. Noise problems have been widely studied and Dr. Landauer considered an example in information theory. Loss of memory of a 'particle' in an information store takes place when thermal agitation is too large in comparison with the height of the potential barrier separating the two alternative positions for the particle. Dr. Landauer mentioned calculations to indicate what was the optimum size for an element in a memory store of given total mass. Most of Dr. Landauer's talk, however, was concerned with friction *per se*. Whenever we have an indistinguishable merging of two streams of particles there must be friction, for without friction the forces on the particles can be described by a field of force only, and this implies that the particle paths are reversible. However, as we wish to 'kill off' the past memory of the particle trajectories, the paths cannot be reversible and so we must have friction in the system. Dr. Landauer argued that if a machine is to make decisions it cannot be acting merely as a table of 'logarithms' in which every situation is allowed for by the designer, but it must contain some processes involving such an irreversible merging of two streams. The work of Szilard on the measuring process was also mentioned; in general, to make a meaningful measurement we must obtain a result independent of the past history of the measuring apparatus, and this necessitates friction in the system, as, for example, the damping of a galvanometer.

The final talk, by Dr. Arnold Burgen (McGill), described up-to-date knowledge of the problem of cellular storage of information. Information is stored in the nucleus of the cell in the compound deoxyribonucleic acid, a complex chain molecule made up of a large number of units or nucleotides. Dr. Burgen argued from chemical data that a simple molecular organism such as a so-called bacteriophage virus (the nucleus of which would contain only one or two molecules of deoxyribonucleic acid) required about 10^4 nucleotides, a cellular organism containing a single chromosome such as a complex bacterium required about 10^6, while the germ cell of a mammal needed about 10^{10} nucleotides. There are four different types of nucleotide which can be arranged in any order on the deoxyribonucleic acid spiral chain. We never find a single chain, but a chain is always accompanied by its complement. Thus an adenine unit on one chain is always cross-linked (by hydrogen bonding) to a thymine unit on the complementary chain, and a guanine unit to a cytosine. This double chain plays an important part in the replication process of the deoxyribonucleic acid and so of the genetic information when the cell divides. It appears that the double helix starts to split from one end and each parent helix then acts as a 'template' to synthesize two daughter helices complementary to the parents. Two separate double helices identical to the parent eventually emerge. This synthesis has recently been carried out in the test-tube, and the postulated mechanism shown to be essentially correct by labelling with tritium and nitrogen-15. Dr.

Burgen went on to discuss the details of how the genetic information was stored in deoxyribonucleic acid, suggesting that it was likely that four bases were needed to represent one piece of information. He finally considered some of the possible effects of a mistake in the replication process, that is, of a mutation. Dr. Burgen's overall aim was to show that there is no difficulty in principle of storing enough genetical and reproductive information in a mammalian germ cell.

A. M. GUÉNAULT

BIOGEOGRAPHY OF SOUTH-EAST AFRICA

A SYMPOSIUM on "The Biogeography of South-east Africa" was held at the University of Natal, Pietermaritzburg, during September 7–8, 1960, under the chairmanship of Prof. S. F. Bush. South-east Africa was taken to include Zululand, Natal and the eastern Cape Province. Particular attention was given to the coastal lowlands, and their affinities with East Africa, but a consideration of the more southern and upland communities made it necessary at times to expand the scope of the symposium to include the south-western Cape.

In south-east Africa the relief, which is in the form of a number of ascending steps, is such that, in general, the inland isotherms tend to run in a north–south direction, parallel to the coast (O. Williams). There is a pronounced difference in temperature between the hot eastern coastlands and the cooler interior highlands, and at the same time temperatures along the coast increase gradually northwards. The coastal plain to the north of Lake St. Lucia (28° S.) experiences a mean July (mid-winter month) temperature of more than 18° C., while areas lying above the 4,000 ft. (1,225 m.) contour experience a mean July temperature of less than 13° C.

Rainfall at the coast ranges from about 30 to 55 in. per annum, and is heaviest at the northern and southern districts of the area considered. Inland, on the seaward-facing escarpments, rainfall is about 70 in. per annum, but on the intervening surfaces it is considerably less. Most of the rainfall is received during summer, but this characteristic is far more pronounced inland than at the coast.

The vegetation of the north-eastern coastal plain is floristically of tropical African affinity (A. W. Bayer and C. J. Ward). Practically every tropical family is represented in northern Zululand, often by the same genera and species as in the tropics. In the case of cosmopolitan families, the Zululand genera are the same as, or closely related to, the tropical African genera. On the other hand, predominantly extra-tropical families are poorly represented in north-eastern Zululand.

The north-eastern vegetation is complex, being made up of a number of different ecological associations, which include mangrove forest, swamp forest, dune forest, coast forest, riverine woodland and savanna. In the region between about 27° 30′ and 29° S., but most noticeably in the Lake St. Lucia area (28° S.), there is a heavy subtraction of tropical species in every type of association. Of a representative 408 species of woody plants occurring in north-east Zululand, approximately 130 do not extend farther south of Lake St. Lucia. To the south of this area of marked thinning out, subtraction is more gradual, the truly tropical element in the flora finally disappearing to the south of East London (33° S.). The number of different plant associations also shows a decline to the south of 28° S.: the swamp forest association does not extend farther south of 30° 45′ S., and the mangrove association terminates at 32° 15′ S.

As the tropical flora becomes impoverished, it becomes admixed with a number of South African species which are not found in north-eastern Zululand.

Regarding sedges, the majority of water-loving species extend from the tropics (where they are distributed across the continent from west to east) through Mozambique and Southern Rhodesia, to the Transvaal, Natal and the Transkei to about 33° 30′ S. (K. D. Gordon–Gray). To the south there is a narrowing of the zone occupied by these plants, so that south of the Natal border only a coastal distribution pertains. In Natal, the majority of the species extend up the river valleys to the foothills of the Drakensberg, but do not appear to cross this mountain barrier into Basutoland and the Orange Free State.

The 'dry-land' sedges follow approximately the same distribution southward, except that they are limited to the drier, and sometimes the higher, land of Southern Rhodesia, and are probably not represented at all on the coastal plain of Mozambique. Annual species, which can more easily overcome the unfavourable season than can perennials, are more widespread from east to west, but they also do not usually extend farther south than 33° 30′ S.

Generally, it may be stated that in this southward 'funnelling' down of the tropical Cyperaceae, there is a marked decrease in the number of species, many tropical species not at present being known from farther south than the St. Lucia area. There is also a marked decrease in the variety of plant forms south of Lake St. Lucia, and a decrease in plant size and inflorescence branching, accompanied by an increase in tufting of the plants and in leaf-blade development.

A temperate sedge flora exists in the south-western area of the sub-continent. This flora extends northwards, particularly along the high ground, and intermingles with the tropical element in Natal. In addition, Natal has its own endemic species.

The freshwater fish fauna of south-east Africa appears to be a very recent fauna, possibly entirely post-Pleistocene, in marked contrast to the temperate fish fauna of the south-western Cape (R. S. Crass). The south-east African fish fauna is clearly tropical in its affinities. Nineteen of the 28 species which occur in the lower Pongolo River (27° S.) extend northwards at least to the Zambesi. Of the remaining 9, 5 are restricted to the eastern Transvaal and the Natal lowlands. These 10 forms may be considered sub-tropical, and are taxonomically closely related to species found farther north.

No strictly freshwater south-east African fish extends south of the Natal border, progressive impoverishment of the fauna occurring southwards. At family-level this trend is illustrated by the fact that out of 14 families found in the Zambesi, only 9 reach the Pongolo (27° S.), 4 the Tugela (29° 15′ S.), and 1 the Umtamvuna (Natal border). This impoverishment does not constitute a true subtraction of the tropical fauna southwards; it is more in the nature of a diffusion gradient which reflects the difficulty experienced by the recent fish fauna in moving southwards across divides between the west-to-east flowing rivers.

Only 13 species occur in the Natal highlands. Of these, 8 are tropical or sub-tropical species, while the remaining 5 may be regarded as temperate, since they are restricted to the cooler highlands. One of these is distributed from the eastern Transvaal to the main Cape watershed, but the others do not extend to the Cape.

The amphibian fauna of north-eastern Zululand is almost identical with that of the lower Zambesi-Beira area, both in its composition and in number of forms (J. C. Poynton). However, in the Lake St. Lucia area, the number of tropical forms drops from 35 to 15. Subtraction farther south is more gradual, but only one form reaches as far as Knysna (34° S.). This subtraction margin is best developed in the coastal lowlands: the tropical fauna becomes markedly thinned out as the land rises from the coast.

South of Lake St. Lucia, the fauna changes from an almost purely tropical to a heterogeneous assemblage, for those tropical forms which pass Lake St. Lucia become joined by a 'South African' fauna, made up partly of sub-tropical Natal-eastern Cape endemics, and partly by members of a temperate fauna[1] centred in the south-western Cape. The subtraction margin of this latter fauna is best developed in the highlands.

The natural distribution patterns of the higher vertebrates have been profoundly upset by human settlement and cultivation, but overall trends are still evident. The tropical element in the avian fauna of north-eastern Zululand is very conspicuous, but there is a marked subtraction of tropical forms in the region of Lake St. Lucia (J. Vincent). Of the 33 species which extend down into Zululand and Natal, but which have no sub-species resident farther south, 17 reach their southern limitation in the St. Lucia region. Elsewhere, subtraction is gradual, but none of these species at present occurs south of Durban.

The majority of tropical mammals occurring in southern Africa show a general distribution pattern which roughly coincides with savanna areas (J. A. Pringle). In the east, this distribution 'funnels down' and extends as a narrow strip along the coast from northern Natal to the south-western Cape. Subtraction of the tropical forms occurs along this 'funnel'. About half the tropical species of Chiroptera and Insectivora do not extend as far south as Natal, the reduction in the number of species apparently beginning in northern Mozambique. On the other hand, most of the Carnivora have a much wider range, and reach Natal. In each of these three groups, the number of tropical species shows a two-thirds reduction between St. Lucia and Port St. Johns (31° 38′ S.), and only a small number in each group extend as far as Cape Town.

The same tendency to 'funnel down' the south-eastern lowlands and extend to the south-western Cape is shown by the tropical rodents (D. H. S. Davis). A southward subtraction is shown in this area. A small 'southern African' element is also represented in this group.

Concerning invertebrates, it appears that the main route of migration from the African tropics is also down the eastern half of southern Africa, but this applies chiefly to groups which have strong and active members which are able to distribute themselves by their own bodily activities, and which are not particularly dependent on the humidity factor (R. F. Lawrence). The position with regard to the humidity-dependent groups is quite different, the tropical and South African faunas having little in common. In the harvest-spiders, for example, the dominant family in tropical Africa is not the dominant family in South Africa, and neither region has a single species of these families in common with the other. The dividing line is the Limpopo region. Again, the South African Onychophora have nothing in common with the tropics, where only one species of a quite different family occurs. In both the harvest-spiders and the Onychophora the relationships of the South African faunas with those of the other continents are greater than with those of the African tropics: their affinities are inter- rather than intra-continental, and latitudinal rather than longitudinal. The cryptic faunas with inter-continental relationships are older, probably very much older, than the groups with intra-continental relationships.

The southern African Diptera also show a marked differentiation into tropical and South African temperate assemblages (H. E. Paterson and B. Stuckenberg). The tropical families tend to funnel down the eastern lowlands, where progressive subtraction occurs. Very few tropical dipterans reach the south-western Cape. The dipteran fauna of the Natal highlands is largely continuous with that of the south-western Cape. This temperate fauna differs strikingly from the dipteran fauna of north-eastern Zululand. The relationships of the temperate fauna are again inter- rather than intra-continental, and latitudinal rather than longitudinal. Some very primitive forms are present in this south-western fauna.

All families of butterflies represented in south-east Africa have a predominance of species in the tropics (K. M. Pennington). Extension of tropical species into South Africa is via the east-coast route, although here the tropical fauna shows a pronounced tapering off. A non-tropical South African fauna is also represented in south-east Africa, particularly in the highlands, where close affinities with the fauna of the south-western Cape are shown.

North-eastern Zululand is very rich in tropical arthropod-borne viruses: in this region 11 viruses have been found to be active (P. Weinbren). From Lake Sibayi (27° 20′ S.) southwards, however, the general virus activity tails off abruptly, and there is evidence of only 3 of the above viruses in Natal. At Knysna, however, 6 of these viruses have been found, producing a pattern which is in contrast to the usual pattern of continued subtraction southwards.

Through the great diversity of groups considered at this symposium, a close conformity of distribution patterns seems unmistakable. The flora and fauna of the northern Zululand plain is truly tropical:

in the north-east, South Africa possesses an almost fully developed tropical East African association. However, in South Africa this association is very definitely restricted to a small area. A well-marked southern 'boundary' is evident in the region of Lake St. Lucia, there being a most pronounced subtraction of tropical species in this region. The factors causing this southward limitation have still to be clarified. The cause of the western limitation of the tropical assemblage inland is explainable in terms of physiography, the ascent to the central plateau causing profound changes in climate; but there are no comparable physiographical changes in the St. Lucia region. There are also no abrupt zonal climatic changes in this region, although it seems significant that the 18° C. mid-winter isotherm crosses the coastal plain in this region, after skirting the western edge of the Mozambique plain. This isotherm is used to divide a tropical from a non-tropical climate in Koppen's classification of climates[2]. Phytogeographical changes in this region are not in themselves sufficient to account for the general changes in the fauna, for although the taxonomic changes in the vegetation are extremely marked, the physiognomic characters of the various plant associations are not conspicuously altered.

Whatever the factors causing the biogeographical changes in Zululand may be, it is evident that the widely held belief that "There is a strong, general reduction of the (tropical) fauna northward into the Sahara and a less strong reduction and some differentiation southward into South Africa[3]" is a serious under-estimation of the extensiveness and importance of these changes. The south-east African plant and animal communities are evidently built up from three quite distinct elements: a tropical complex entering from the north, a warm temperate complex entering from the south-west, and a relatively small complex indigenous to the area. The area clearly shows the characteristics of a transition zone, and this symposium brought into particular prominence the fundamental difference between the flora and fauna of the African tropics and the flora and fauna centred in the south-western Cape. The recognition of the south-western Cape as a biogeographical region distinct from the Ethiopian region is indicated by all the groups which are less recent than the contemporary birds and mammals, the members of which have the added advantage of homoiothermy. Birds and mammals traditionally form the zoogeographer's stock-in-trade; but a proper assessment of the status of the southern temperate region of Africa is only possible if these groups are seen in a balanced perspective.

J. C. Poynton

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[1] Poynton, J. C., *S. Afr. J. Sci.*, **56**, 307 (1960).

[2] Köppen, W., *Grundriss der Klimakunde*, second edit. (Walter de Gruyter and Co., Berlin, 1931).

[3] Darlington, P. J., *Zoogeography: the Geographical Distribution of Animals*, 433 (Wiley and Sons, New York, 1957).

HIGHER EDUCATION IN THE UNITED STATES

Dr. J. B. CONANT has recently examined some of the ambiguities between school, college and university in the United States and compared the American approach to the university with that of certain European countries (*Proc. Amer. Phil. Soc.*, 104, No. 6; December 15, 1960).

Conant believes that the comparison between the large numbers of American youth attending a college or university and the small numbers enrolled in European universities is misleading. A European university is essentially a collection of professional schools. If one compares the percentage of an age-group attending universities in a European nation and those attending American postgraduate institutions, the difference is not very striking.

The road to the university in Europe does not pass through the equivalent of an American college. Instead, there are essentially pre-university schools for those who aim at university education. They offer a stiff academic course of eight or nine years in length centred on either: (*a*) Latin and Greek, or (*b*) Latin and a modern language (or two) and (*c*) modern languages, science and mathematics. In each of the German States, and in each of the Swiss cantons, there are three types of pre-university schools, each preparing students to pass examinations for a State certificate which, in turn, provides admission to a university anywhere in the country.

The American liberal arts college is in the nature of an accident. During the nineteenth century the programmes which were offered for the first degree in arts or science, or in letters, were expanded to include a number of practical subjects. This fact makes it difficult to explain to a European what is meant by an American college. Within a university, too, there is a great variety of four-year programmes of study which may be entered directly by high-school graduates.

An American college is possibly intermediate between a high school and a 'university' in the European sense of the word. For the most part, those who become lawyers now study three or four years in an undergraduate department of a university, or in a four-year liberal arts college before they enrol at a law school; the same is true of medical students. On the other hand, engineering schools never became postgraduate schools. As a consequence, high-school graduates enter directly on their professional training if they want to become engineers. With so many complications in mind, it is hard to define without ambiguity the relation of a college to the education of members of the learned professions.

There is another ambiguity inherent in the American pattern of higher education. The standards of admission to the American colleges and universities are so varied as to, make it impossible to describe them accurately.

Standards for the first degree are almost as diverse as the standards of admission; the mere holding of a bachelor's degree has almost no significance. Yet no one would publish a rank list of institutions or even be willing to be quoted publicly to the effect that a straight '*A*' record in one college has about the same meaning as a '*C*' record in another. In the United States, there is a hierarchy of institutions granting the first degree and a complete conspiracy of silence as to the existence of such a hierarchy.

On entrance tests, Conant recommends that leading professional schools of law and medicine and graduate schools of arts and sciences might require their candidates to pass a set of examinations which would demonstrate a mastery of academic subjects somewhat comparable to that required for entrance into a European university. The requirements might be as follows: a good reading, writing and speaking knowledge of at least one foreign language; a knowledge of mathematics as far as the calculus; a knowledge of physics, chemistry and biology at the freshman college-level; the ability to write a competent essay; a knowledge of American history and political constitutions at a sophisticated level; and a knowledge of English and American literature at a corresponding level.

The variety of colleges prevents any uniform set of standards for admission. Therefore, the colleges could not, even if they desired, greatly influence the high-school curricula. But the relatively homogeneous nature of the graduate and professional schools makes the formulation of standards possible. The effect of this formulation would influence both colleges and high schools.

CIRRHOSIS OF THE LIVER IN FRANCE

AN investigation was recently undertaken by the nutrition department of the Institut national d'Hygiene, Paris, into the role of alcohol in the ætiology of cirrhosis of the liver in France (*W.H.O. Chronicle*, 14, December 1960).

Cirrhosis of the liver is relatively more common in France than in the rest of Europe; the death-rate in 1956 was the highest in Western Europe (32·5 per 100,000 inhabitants, as against 2·6 in England and Wales and 13·6 in the Federal Republic of Germany). For many years the medical profession in France has attributed the high incidence of the disease to the excessive consumption of alcohol, and statistics from hospitals agree that 80–85 per cent of cirrhoses are alcoholic in origin. But the medical profession has been accused of prejudice, and wine merchants have put forward the argument that in a population of whom 80 per cent are drinkers, it could be maintained that practically all illnesses are alcoholic in origin.

The survey was designed to answer five questions: did persons believed to have cirrhosis of alcoholic origin drink more before their illness than random controls of the same age, same sex and same social environment without cirrhosis; were they less well nourished (that is, is cirrhosis due to alcohol or to an associated nutritional deficiency); is the reduced food intake so often seen when cirrhosis becomes decompensated a cause or a consequence of the decompensation; can the amount of alcohol that causes cirrhosis be determined; and what part does wine play in the alcoholism of cirrhotics?

The survey was carried out in hospitals in Paris, Marseilles, and Nantes, all patients with alcoholic cirrhosis being questioned and a non-cirrhotic control being chosen at random in the same department for each patient questioned. The physical activity at work and outside work, the height and the usual weight before cirrhosis began were recorded, and a detailed food questionnaire was devised to establish the daily food intake before and after the onset of the disease, as well as the quantity of alcohol absorbed daily. In addition, questions were asked to reveal the social background about the patients' family and parents, income, living conditions and standard of living.

In all, 116 cirrhotics and 116 controls were compared. They did not differ significantly in age, physical activity, weight, or calorie or protein intake; but the alcohol intake of the cirrhotics was found to be approximately 2·5 times greater than that of the non-cirrhotics. Before they fell ill, the cirrhotics has been just as well nourished as the others; consequently, it was concluded that cirrhosis cannot be attributed to dietary deficiencies.

Only one out of the 116 patients with cirrhosis said that he had consumed less than 80 gm. of alcohol (that is, less than 1 litre of wine at 10°) daily. From this it may be inferred that alcoholic cirrhosis appears only when consumption of alcohol is greater than 1 litre a day. But 50 out of the 116 controls consumed 80–160 gm. of alcohol a day (1–2 litres of wine), and it was only in the category from 160 gm. of alcohol (2 litres of wine) up that controls tended to decrease in number (7 out of 116), while more than half the cirrhotics (64 out of 116) came within this range.

It seems reasonable to infer that cirrhosis is likeliest when the intake is between 2 and 2·5 litres of wine a day (172–212 gm. of alcohol). Cirrhosis can occur when it is 1 litre or more, but when it exceeds 2 litres the probability of its occurrence is much increased.

Is decreased food intake a decompensating factor in cirrhosis? Apparently not to any marked degree: in 88 cases out of the 116 it failed to cause decompensation; thus even at this late state the nutritional deficiency is only associated, not causal.

Finally, the survey established that wine represented 80–90 per cent of the alcohol ingested.

ELECTROLYTIC JUNCTION WITH AMPLIFYING PROPERTIES

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IN previous papers[1,2] it has been shown that the rectification of alternating electric current at a junction of two electrolytes is possible. This is analogous to the same effect at a junction of n- and p-type semiconductors. It is to be expected that other effects in semiconductors, for example, the amplification of electric current, have analogies in electrolytes. The purpose of this article is to report that this has been achieved.

The electric circuit is similar to that used with Ovitron amplifiers[3]. The essential difference between the Ovitron and the device reported in this article is that the active parts of Ovitron amplifiers are metal-metal oxide electrodes, and here electrolyte-electrolyte junctions.

The device (Fig. 1) consists of two rectifying elements of the type described earlier[1], turned face to face in such a way that, in the ideal case, alternating current in the main circuit could not pass through. Each of the rectifying elements consists of two ion-exchange membranes : cation in H^+ form and anion in OH^- form ('Zerolit permaplex' *C*-20 and *A*-20, respectively, United Water Softeners, Ltd., London). A thin, perforated polyvinylchloride sheet (0·025 mm. thick) is placed between the membranes to reduce the active surface of the rectifier. Ion-exchange membranes are always wet, due to sheets of filter paper soaked in pure water, and attached to the membranes. The whole device is held together with three pieces of graphite, which also serve as electrical connexions.

Electric impulses (direct or half-wave rectified current) in the second, 'impulse' circuit pass through rectifying elements in forward direction. A full-wave rectifying tube, used as double diode, and a high resistance in that circuit prevents the flow of alternating current from the main circuit. The impulses from the impulse circuit cause the rectifying elements to become 'conductive', and the amplification of the current is observed as the result. The current in both circuits is measured on milliammeters (I_1, I_2), and the current in the main circuit is also followed on a cathode ray oscilloscope screen. Alternating current (50 cycles per sec.) is applied in the main circuit. The pulsating voltage in the impulse circuit is obtained from alternating current of the same frequency and phase as that in the main circuit by passing through the diode. If the current passing through the main circuit has not exactly the sine form, the milliammeter does not show the right value. Therefore the curves on the cathode ray oscilloscope screen are photographed and the exact values of the current obtained by integration.

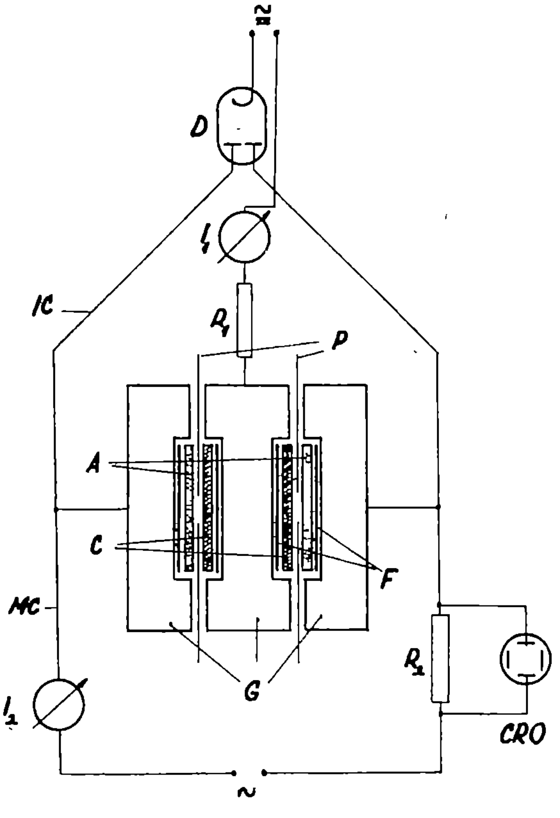

Fig. 1. Schematic presentation of device: *IC*, impulse circuit; *MC*, main circuit; *A*, anion exchange membranes; *C*, cation exchange membranes; *G*, graphite pieces; *P*, polyvinylchloride sheets; *F*, sheets of filter paper; R_1, R_2, resistances; I_1, I_2, milliammeters; *D*, double diode; *CRO*, cathode ray oscilloscope screen

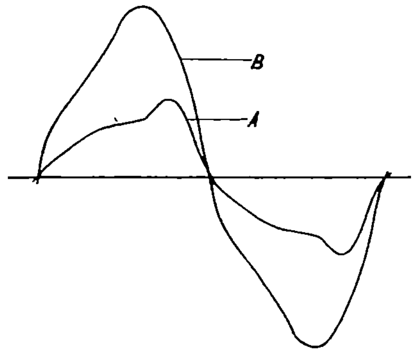

Fig. 2. Typical curve with direct current in impulse circuit: *A*, current in the main circuit before impulse is applied; *B*, current in the main circuit during the impulse

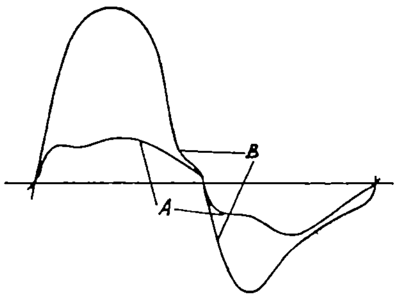

Fig. 3. Typical curve with rectified sinusoidal alternating current in impulse circuit: *A*, current in the main circuit before impulse is applied; *B*, current in the main circuit during the impulse

In each experiment, first the current in the main circuit is determined (it is different from zero, because of non-ideal rectification). Then, the impulse circuit is switched on, and immediately the current in the main circuit is determined again. The impulse circuit remains closed until the current in the main circuit stabilizes (1 min.). The stabilized current is determined, the impulse circuit cut off, and the current in the main circuit, in 'unconductive' state, is determined again.

The amplification is obtained by means of the equation :

$$\text{per cent amplification} = \frac{i_m - i_s}{i_s} \times 100$$

where i_m = the increase of current in the main circuit, and i_s = the current in the impulse circuit.

When the direct voltage in the impulse circuit is applied, the amplification, immediately after closing this circuit, is about 60 per cent and, after stabilization, about 100 per cent. In the case when pulsating voltage is applied, amplifications of 45 per cent and 80 per cent are obtained. Typical curves with direct and pulsating current are shown in Figs. 2 and 3.

Curves *A* in Figs. 2 and 3 represent non-ideally rectified alternating current. Both halves of the cycle are equally distorted, because the current passes through two rectifying elements, turned face to face (Fig. 1). The current increases symmetrically in both halves of the alternating-current cycle if direct current is applied in the impulse circuit over the full alternating current cycle (curve *B*, Fig. 2). If the impulse is a half-wave rectified sinusoidal alternating current of the same frequency and phase as that in the main circuit the situation is different (Fig. 3). The first half of the main circuit alternating-current cycle is that in which the impulse is applied, and an increase of the current is observed. But an increase is also observed in the second half of the cycle, where no impulse is given (curve *B*, Fig. 3). In such cases evidently the amplification has a certain time-lag. This 'frequency-response' of such systems is very interesting, and it is the object of further investigations.

In both cases (direct or alternating current impulse), the current in the main circuit not only increases but also the shape of the curve approaches the sinusoidal form. This is strong evidence that the amplification is a consequence of a diminished rectifying action, caused by an electrical impulse, which impels the ions toward the rectifying interfaces.

We wish to thank Dr. I. Hruška for helpful discussions of this work.

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SOME FACTORS INFLUENCING THE FOOD-CHAIN TRANSPORT OF RADIOACTIVE MATERIALS INTO COW'S MILK

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VARIATIONS in the radioactive contents of milk have been observed and have been variously attributed to seasonal influences, changes in feeding practices, varying contamination-rates, etc.[1–4]. No detailed explanation of the fundamental principles underlying these variations has yet been presented. The following approach provides a method by which such variations can be explained.

The discussion is based on the fact that cows are fed on grass, hay, etc., which, prior to its consumption by the animal, has been exposed to radioactive contamination over a period of time. The general applicability of the proposed method will be shown in three model cases which illustrate three combinations of basic conditions :

I. (*a*) Constant feeding conditions throughout the year ; (*b*) contamination of the fodder with radioactive material at a constant rate. II. (*a*) Constant feeding conditions throughout the year ; (*b*) contamination with radioactive material at a varying rate. III. (*a*) Seasonal variations of feeding conditions ; (*b*) contamination with radioactive material at a constant rate.

Two assumptions are made in order to simplify the presentation : (*A*) Contamination of milk is assumed to be essentially proportional to the contamination of the diet of the cattle. (*B*) Contamination of the diet is assumed to be essentially proportional to the product of the rate of contamination by the time of exposure.

Other factors which influence to some degree the contamination of fodder and milk are not introduced in the discussion of the three model cases because they do not affect the basic considerations.

In the graphic presentation of the three model cases I, II and III (Figs. 1 and 2) twelve successive conditions *A*–*L* are shown for each of these model cases. In each sub-graph of this presentation the abscissa gives the time-scale while the ordinate

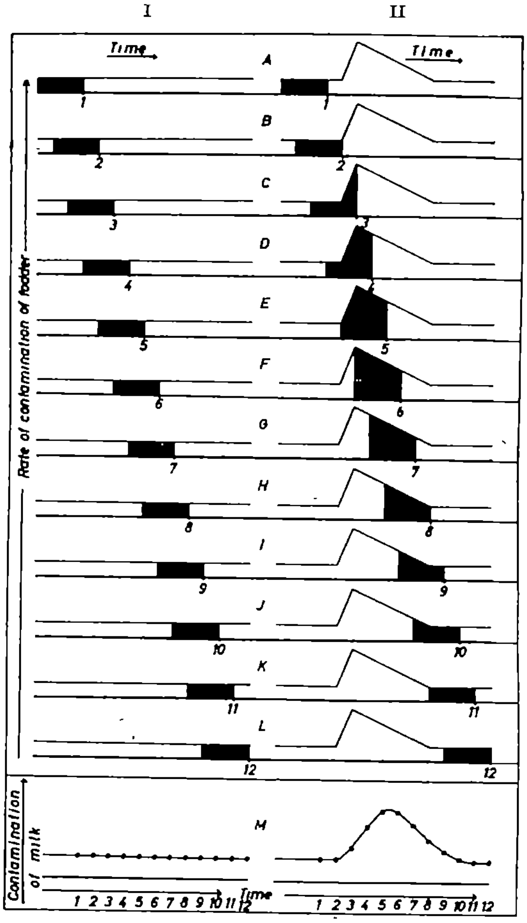

Fig. 1

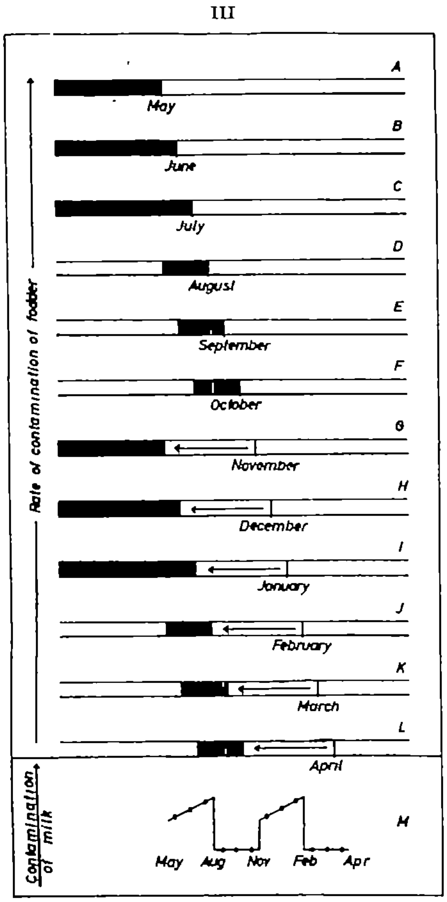

Fig. 2

indicates the rate of contamination. The total amount of radioactivity supplied with the grass to the cattle at times 1–12 is shown by the shaded areas in sub-graphs *A–L*. Sub-graphs *M* show the resulting contamination of the milk as a function of time.

The graphic presentation in model case I (constant feeding conditions, constant rate of contamination) shows clearly that the contamination of the milk remains constant, as shown in sub-graph (I) *M* of Fig. 1.

In model case II (constant feeding conditions, variable rate of contamination) the rate of contamination is assumed to rise gradually to a maximum at time 3, slowly returning to 'normal' at time 8. The cows feed on grass contaminated by amounts of radioactivity equal to the shaded areas of sub-graphs (II) *A–L*, respectively. At time 3 they consume grass which has for a considerable time been exposed to contamination at a low rate and for some time to contamination at a high rate. The total accumulated contamination of the grass ingested by the cattle at time 3 for that reason is somewhat higher than at times 1 or 2. Thus contamination of milk will increase until a maximum is reached between times 5 and 6. Later, milk contamination decreases again, and at time 11 it reaches its original value.

The result is demonstrated in sub-graph (II) *M* : the maximum rate of contamination at time 3 causes a maximum of milk contamination at times 5–6.

Model case III, shown in Fig. 2, illustrates a typical case approximating conditions in a country where the feeding conditions change with the seasons. The rate of contamination of the fodder is assumed to be constant.

Sub-graphs *A–L* illustrate the following pattern of feeding conditions : cows are kept in a barn until May. During this time they are fed on hay harvested during the previous year. In May they are led to the pasture (sub-graph *A*). The grass they feed on from May until July has not been cut since November of the previous year and has been contaminated at a constant rate (sub-graphs *A–C*). By August the cattle return to pastures which have already once been grazed in spring. They start eating grass grown since May and thus exposed to the constant rate of contamination for three months only. They feed on this pasture from August until October (sub-graphs *D–F*). After this time, they return to the barn and are again fed on hay harvested between May and July, as indicated by the arrows (sub-graphs *G–I*), and between August and October (sub-graphs *J–L*). respectively.

Sub-graph *M* of Fig. 2 shows the resulting contamination of the milk from cattle fed under these conditions. It shows that two distinct peak periods of radioactive contamination must exist during the year, even though the rate of contamination remains constant.

The above analysis has not taken into account the influence of variations in food composition. In practice, in many countries of the northern hemisphere, cows are fed on mixtures of hay and concentrates from November to April.[1] These concentrates, consisting of oil cake, etc., contain as a rule very little contamination. For this reason the November–January peak-level should be lower than the May–July peak-level, and the February–April values should be lower than the August–October values. Hay, on the other hand, is not always fed in the sequence in

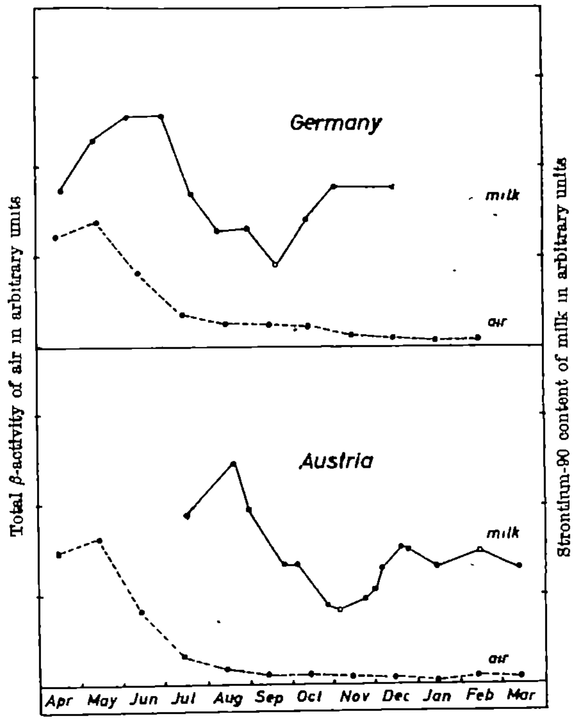

Fig. 3. Radioactivity of milk and air 1959/60

which it was harvested, and this will tend to level off differences between the values for the periods November–January and February–April. In addition, we have to consider the influence of varying deposition-rates superimposed on the seasonal variations of feeding conditions.

In order to demonstrate the applicability to actual conditions of the theoretical considerations discussed in the three model cases, Fig. 3 is presented. It shows the results of measurements of strontium-90 in milk from German and Austrian sources carried out in 1959 and 1960 at the Bundesforschungsanstalt für Milchwirtschaft, Kiel, and at the provisional laboratory of the International Atomic Energy Agency, respectively. These values are shown in relation to results of measurements of radioactivity in air[5,6].

In Germany and Austria conditions are comparable to those considered in model case III. The reasons for the delay between the appearance of a maximum of radioactivity in air and a corresponding maximum of contamination in milk have been discussed in model case II.

Similar results have been reported from sources in the United Kingdom, Canada and the United States[7-10].

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DIQUAT AS AN ARBORICIDE

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SODIUM arsenite has been widely employed as an arboricide and herbicide, but in various countries its use is being progressively restricted or prohibited. Investigations are being carried out at the West African Institute for Oil Palm Research with a variety of herbicides and other chemicals to find an alternative to sodium arsenite for poisoning old oil palms prior to, or concurrently with, replanting. So far diquat, a new desiccant and herbicide first described by Brian *et al.*[1], is the only one that has been found to be consistently effective and it is believed that this is the first record of its use as an aboricide.

The palms used in the trials were planted in 1939 and had attained a height of 25–30 ft. During the rainy season diquat was poured into a small, slightly downward sloping hole about 8 in. deep, punched 2–3 ft. from the ground in the trunk of the palm with a crowbar. The hole was then almost filled with water. Two separate trials were carried out, four palms being treated at each dosage-rate. Diquat was introduced at the following rates of active ingredient per palm:

First trial: 20 gm., 50 gm.
Second trial: 10 gm.; 20 gm.; 40 gm.; 60 gm; 10 gm., followed by a further 10 gm. after three weeks.

The effect at rates of 40 gm. and more was very rapid. Within two weeks of poisoning, all palms which had received these high rates had suffered almost complete desiccation of the older fronds and in some cases desiccation had proceeded as far as the central spear. At the 20-gm. rate the symptoms appeared more slowly, but followed the same course. It was evident that 10 gm. is too little to kill an adult palm and two applications of 10 gm. at three-week intervals were less effective than the single application of 20 gm.

After six weeks the fronds in most of the affected palms had collapsed basally and remained cloaking the stem; in other palms the fronds dried out in their normal position with some fracturing along their length. In all but one palm the spear remained erect but dried out. This is in contrast to the appearance of palms poisoned by sodium arsenite, where the spear usually falls out and the trunk collapses at any point along its length within two months of poisoning[2]. The collapse of the trunks of palms poisoned by diquat is, however, only a matter of time.

One palm receiving 50 gm. diquat was felled three weeks after poisoning. The expanded fronds were all dead; but the spear and one immature frond were still green. Internally the trunk appeared drier than normal with some pink discoloration of tissues around and below (but not above) the point of injection. A longitudinal section through the growing point showed no abnormal symptoms at that time. At the centre of the rachis of each dead frond, a pinkish brown discoloration was evident which was more advanced distally; it appeared to have started in the pinnæ and spread backwards. Transverse sections of some leaf bases showed slightly discoloured, water-soaked areas.

Another palm which had received 50 gm. diquat, was felled and examined after five and a half weeks; all the pinnæ were desiccated, but there were green areas in the spear and in an immature frond. A longitudinal section through the crown showed that the pinkish brown discoloration had spread to the leaf bases. The entire central portion of the rachis was coloured brown and there were roughly circular areas infected by a black rot. The rot appeared to be invading the spear in an irregular manner, some portions remaining green, others becoming desiccated and brown. The rot had penetrated to within a foot above the growing point, and the whole crown emitted a slightly putrid odour.

Three palms in the second trial were felled two months after poisoning. Symptoms in those which had received rates of 20 gm. diquat and more were similar, but the development of symptoms was more advanced at the higher rates. A soft, wet rot was observed in the trunk, affecting half the area near the point of injection. The tissues between the vascular bundles had completely broken down and this area was demarcated by a dark brown line. The tissue outside this area was obviously not normal, some tissue breakdown having occurred and the colour being a more yellowish pink than in a normal palm. The growing point was found to be completely destroyed by a wet, putrid rot. There was evidence of wide translocation of the poison; sectioning of the roots revealed brown to black discoloration of the vascular strands and a breakdown of cortical tissue.

At present this method of poisoning is expensive, but diquat has proved more effective than sodium arsenite, which, in West Africa, has given erratic results, except at much higher rates than those found necessary in Malaya[2]. The comparative safety with which diquat may be handled and used is an important point in its favour.

The purpose of this article is to direct attention to the arboricidal properties of diquat; the satisfactory results obtained with it for poisoning oil palms suggest that its use in this way may be of wider application. The samples used were kindly made available, as 'Reglone', by the manufacturers, Imperial Chemical Industries, Ltd.

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'ACTIVE CENTRE' STUDIES IN TRYPSIN

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THE understanding of the mechanism of enzyme action presupposes a knowledge of structural elements of both substrate and the enzyme. This is relatively easy for substrates, which are usually of low molecular weight, but has not yet been achieved for any enzyme because of the complexity of the protein molecule. The essential structural requirements of the enzyme are referred to as its 'active centre'. They should be distinguished from those structural elements of the enzyme necessary for the preservation of its native configuration and not participating directly in the binding or activation of the substrate.

In the particular case of trypsin, its substrate requirements were believed to have been solved earlier[1]. Employing synthetic polypeptides, it was reported that trypsin will only catalyse the hydrolysis of bonds involving the carbonyl groups of the basic amino-acids arginine and lysine. This observation, though essential, did not contribute to the knowledge of the active centre of trypsin. Paradoxically, most of our information about the active site came from the discovery that trypsin and its close analogue chymotrypsin act on substrates bearing no apparent similarity with the above-mentioned ones. These are nitrophenyl acetate (NPA)[2] and di*iso*propylfluorophosphate (DFP)[3]. The latter reacts with trypsin and chymotrypsin in a mole-for-mole ratio to give stable inactive products, the *p*H curve of inactivation being similar to that of the enzymic activity. With NPA, chymotrypsin is inactivated, yielding a monoacetyl derivative. At lower *p*H values, for example, *p*H 5–6, the acetyl group is stable in aqueous solution and reactive towards hydroxylamine[4,5], while at *p*H 7–8 hydrolysis occurs, yielding acetate ions and reactivated enzyme. The group reactive towards NPA could also be readily acylated by various acid anhydrides and acid chlorides employed in an equimolar ratio. With trypsin, hydrolysis of NPA is observed, but no inactivation of the enzyme takes place.

The foregoing observations have implicated the seryl and the imidazolyl residues of trypsin as possible functional groups involved in enzyme action. Another approach to the problem of the active centre has been carried out with the aid of reagents specific for various functional groups in proteins. Acylation of amino-groups left the enzyme still active, while esterification of the carboxyl group gave only partially active protein. However, iodination of the phenolic groups and sulphation of the hydroxyl groups rendered the enzyme completely inactive.

This approach lacks the desired specificity, as all proteins possess essentially the same limited number of reactive groups as part of their structure. Accordingly, the key to their specificity is to be sought in the spatial distribution of these groups as conditioned by the general geometry of their molecule, the charge distribution and its fluctuation, and the possibility of resonance and chelation with the substrate. Hence, it is possible to influence the properties of an enzyme through modification of these factors by either blocking accessible reactive groups or introducing new groups into its molecular structure. In this Laboratory such a broader view of chemical modification was taken, since each derivative formed, even though it may still retain enzymic activity, has acquired a new set of properties which differentiate it from the parent enzyme, or any other of its derivatives. For example, the series of acyltrypsins, obtained through reaction of the native trypsin with seven anhydrides of mono- and di-carboxylic acids, differed in their proteolytic and esterolytic activities[6], Michaelis–Menten constants, *p*H optima and dissociation constants with their inhibitors, ovomucoid[7] and serum inhibitor[8]. All these parameters of enzyme action depend directly, one presumes, on the interaction between substrate and enzyme on the level of its active site. Strikingly enough, in some instances, far greater differences were observed in trypsin properties not related to the active site but to its general molecular dynamics. These are: stability towards acid, alkali and urea denaturation and self-digestion[9], ease of reversibility of heat denaturation[10], electrophoretic and ultracentrifugal properties and isoelectric points[7]. As the reaction under the conditions employed causes mainly acylation of the amino-groups, it is obvious that the reacted amino-groups are not indispensable for enzyme action. As they modify it, however, they could be located peripherically to the active centre. A varying area of tryptic active site would also account for the repeatedly observed different effects of chemical modification, when several substrates are used. Most striking is the insensitivity of the active site of trypsin to overall charge distribution on the protein, as the isoelectric point can be changed from 10·8 of the native enzyme to about *p*H 4 of succinyltrypsin, without greatly affecting its parameters of enzyme action.

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Earlier work in this Laboratory with citraconyl and itaconyl trypsin has shown that their proteolytic, but not esterolytic, activity increased on incubation at various *p*H values. Obviously, a direct modification of the active site was involved. This prompted us to investigate the early phases of acylation and its effects on proteolytic activity, which were neglected in most previous work. It was observed that the proteolytic activity of trypsin decreases radically, sometimes even completely, within 3–8 min. on addition of various anhydrides, a slow reactivation taking place on further incubation of the acetylating mixture[11]. Both, inactivation and reactivation, are strongly *p*H dependent. Immediate reactivation can be obtained with hydroxylamine.

Acetylation of Trypsin with Acetic Anhydride

Acetylation in sodium acetate. In the first series of experiments the acetylation was carried out by a slight modification of the customary method[12], employing 0·5 *M* sodium acetate buffer rather than a half-saturated solution. The acetylation mixture was maintained at 0° C. and kept uniformly stirred by means of a magnetic stirrer. The proteolytic activity was determined on urea-denatured hæmoglobin at various times after the addition of anhydride. The *p*H of the hæmoglobin substrate was adjusted to give a final *p*H of 7·8–8·1 on the addition of the acetylating mixture. The concentration of the split products was determined from the absorption at 280 mμ using a Zeiss *PMQ*II spectrophotometer for the activity measurements.

Results presented in Fig. 1 show that there is a slight loss of activity even on addition of one equivalent of acetic anhydride per mole of trypsin.

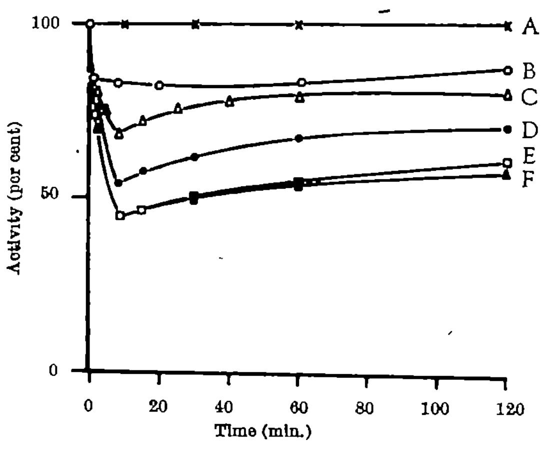

Fig. 1 Acetylation of trypsin with various amounts of acetic anhydride. 5 mgm. of trypsin in 10 ml. of 0·5 *M* sodium acetate was acetylated at 0° C. with: *A*, 80 equivalents acetic acid; *B*, 1 equivalent acetic anhydride; *C*, 4 equivalents; *D*, 20 equivalents; *E*, 40 equivalents, and *F*, 60 equivalents

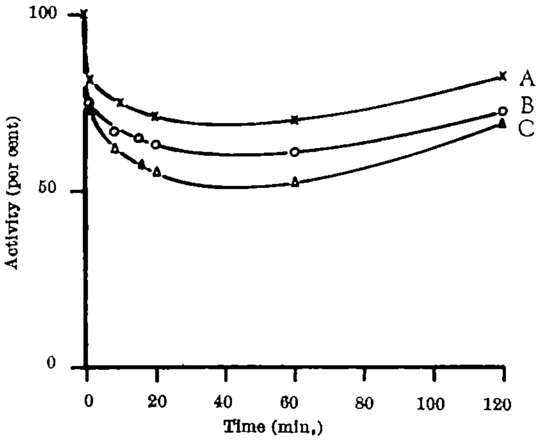

Fig. 2. Effect of buffer concentration. 5 mgm. of trypsin in 10 ml. of solution was acetylated with 40 equivalents of acetic anhydride containing: *A*, 20 mgm.; *B*, 100 mgm.; *C*, 200 mgm. sodium acetate

Maximum loss of activity occurs when 40 equivalents or more of anhydride are employed. There is a serious loss of activity within 1 min. of acetylation. At about 8–10 min. of acetylation, the activity reaches a minimum and begins thereafter to increase. Control experiments show that there is no change in activity on addition of 80 equivalents of glacial acetic acid to the enzyme solution, nor is there a change in activity if acetic anhydride is added to the substrate prior to the addition of the enzyme in amounts corresponding to that present in the enzyme sample used. Customarily, acetylation is performed in half-saturated sodium acetate at 0° C. employing 1·2 ml. of acetic anhydride per gram of trypsin. In Fig. 2 is shown the decrease in activity as the buffer concentration is increased during acetylation. The observed effect is probably due to the increasing alkalinity of more concentrated solutions.

The results of repeated additions of small amounts of anhydride are listed in Table 1. At the first few additions there is a decrease in activity followed by a slow increase. The effect is smaller, however, on second addition, and practically disappears on the third and the fourth. The acetylation of the enzyme during the first addition has an obvious protective effect. The substitution of calcium acetate for sodium acetate did not, however, change the general pattern of the reaction. Similarly, neither did the acetate salts of potassium, magnesium, manganese, barium, aluminium and lead, provided that the *p*H of the acetylating mixture was in the range of 5·5–6·0.

Acetylation at constant pH values. The change of the *p*H of the buffer had, however, far greater effects, if, for example, 0·5 *M* buffers of sodium and potassium carbonate, bicarbonate and borate were used. Under these conditions the proteolytic activity approached zero within 1–3 min. on addition of

Table 1. REPEATED ACETYLATION OF TRYPSIN WITH FOUR EQUIVALENTS OF ACETIC ANHYDRIDE

Time (min.)	Per cent activity			
	First addition	Second	Third	Fourth
0	100	74	76	77
8	77	71	71	76
20	74	73·5	72·5	76·5
30	76·5	76	76	77

anhydride, and, as before, a reactivation took place on incubation of the acetylating mixture[11]. To be able better to explore the dependence on *p*H of both inactivation and reactivation, an automatic titrator was employed in subsequent experiments. The enzyme was dissolved in distilled water and the titrator started at the same time that 65 equivalents of anhydride were added. The *p*H was maintained at the preset value throughout the experiment. The lack of excess buffers permits easier readjustment of *p*H during reactivation studies. In Table 2 is shown the extent of the maximum loss of activity at various preset *p*H values. It is evident that as the *p*H is lowered from 9 to 5 the extent of inactivation decreased from 100 per cent to about 40 per cent. Calcium ions do not have any protective action when acetylation is carried on under the above conditions. The maximum reactivation observed so far amounts to about 50–60 per cent of the native trypsin and to about 80–90 per cent of control samples of acetyltrypsin obtained after exhaustive dialysis and lyophilization. The discrepancy may be due to the fact that trypsin is in equilibrium between a denatured and native form, the latter yielding a fully active acyl derivative while the reversible denatured form becomes irreversibly denatured on acetylation[12].

Table 2. ACETYLATION AT DIFFERENT *p*H VALUES

*p*H	Activity (per cent)			
	0 hr.	8 min.	4 hr.	24 hr.
5	100	62·9	72·5	72·5
6	100	35·1	42·8	42·8
7	100	25·2	44·3	44·1
8	100	0	44·8	44·1
9	100	0	42·7	40·8

The spontaneous reactivation progresses slowly on incubation, but an almost instantaneous reactivation takes place on treatment with hydroxylamine. After 8 min. of acetylation 4 equivalents of hydroxylamine hyrochloride per equivalent of anhydride were added to the acetylating mixture. The hydroxylamine solution was first brought to the *p*H at which the activation was to be carried out and the *p*H of the caetylating mixture controlled by the automatic titrator. Reactivation was measured after further incubation of 8–10 min. at 0° C. The percentage of reactivation with hydroxylamine as a function of *p*H is illustrated in Fig. 3. Curve *A* shows the extent of activation obtained by spontaneous reactivation without hydroxylamine on incubation of the acetylating mixture at the indicated *p*H values for the same length of time. It can be seen that above *p*H 7 the spontaneous reactivation is not measurable within the incubation period of 8 min. On the contrary, with hydroxylamine (curve *B*) maximum reactivation occurs in the *p*H region of 8–9·5. (Chymotrypsin behaves similarly to trypsin, and its acetylation at *p*H 8 or above results in a reversible inactivation. Maximum reactivation with hydroxylamine takes place in the region of *p*H 7·5–8·0.) This dependence on *p*H of the reactivation is indicative of the presence of O-acetyl groups which react with hydroxylamine in this *p*H region[14]. The spontaneous acid hydrolysis confirms this suspicion. Under the same conditions aliphatic ester, such as ethyl acetate, did not react with hydroxylamine until above *p*H 11, while NPA reacts already at *p*H 6·5 and above. The group present in the enzyme has to have a reactivity intermediate to those of the above two compounds. The spontaneous hydrolysis is far more sensitive to acid than to alkali catalysis.

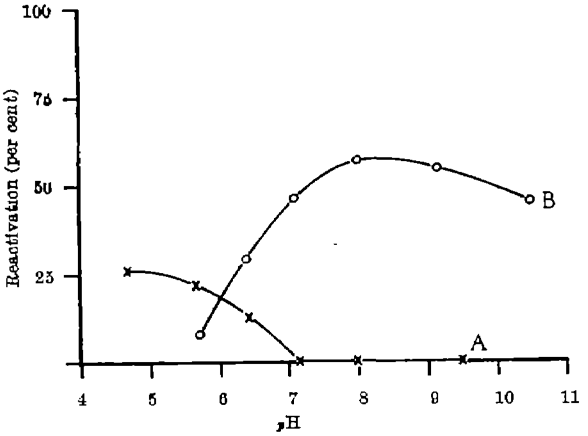

Fig. 3. Reactivation of acetyltrypsin at different *p*H values for an incubation period of 8 min. *A*, spontaneous; *B*, with hydroxylamine

It appeared possible that the imidazole moiety of histidine residues of trypsin can undergo acetylation and spontaneous deacetylation. It is known that acetyl imidazole has a high absorption in the ultraviolet region of 245–260 mμ[15]. Trypsin has 3 moles of histidine per mole of enzyme, and if these groups are undergoing acetylation and hydrolysis, then there should have been a marked increase and a slow decrease in the absorption spectrum at 245 mμ. In Fig. 4 it can be seen that the effect of addition of acetic anhydride to a sample of trypsin (curve *B*) resembles the course of hydrolysis of the acetic anhydride in the same buffer 0·4 *M* borate (curve *A*). The curves were corrected for the absorption of the protein itself. At the same time curve *D* shows an

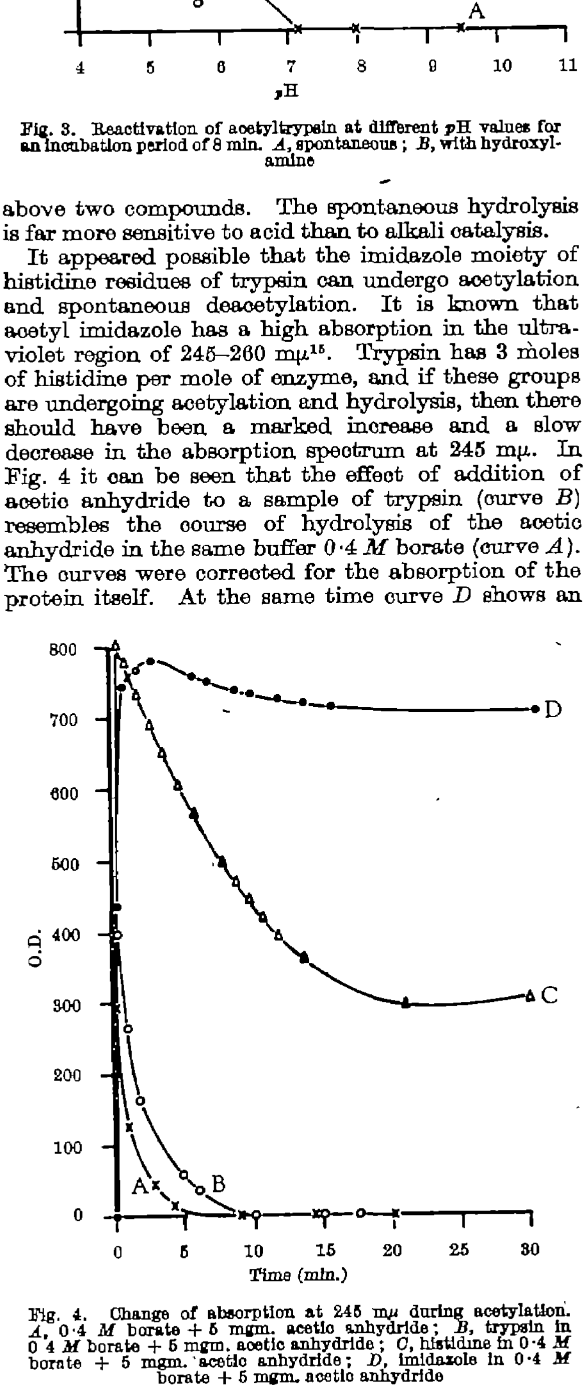

Fig. 4. Change of absorption at 245 mμ during acetylation. *A*, 0·4 *M* borate + 5 mgm. acetic anhydride; *B*, trypsin in 0·4 *M* borate + 5 mgm. acetic anhydride; *C*, histidine in 0·4 *M* borate + 5 mgm. acetic anhydride; *D*, imidazole in 0·4 *M* borate + 5 mgm. acetic anhydride

increase in absorbance from zero to 0·795 on the addition of the same amount of acetic anhydride to a sample of imidazole in a concentration corresponding to that of histidine in the trypsin sample. Curve *C* indicates the change in absorption of histidine under the same conditions; both these curves are quite different from those obtained with trypsin. It appears, therefore, very unlikely that an imidazole group is involved in the acetylation causing the inactivation of the enzyme, though a role of the group in deacetylation[16] cannot be excluded.

Discussion

Tracer experiments show that $DI^{32}P$ is attached to the hydroxyl group of a serine residue in the reaction causing inactivation of either trypsin or chymotrypsin[17,18]. Kinetic considerations led[19] to the postulation that the same group is acetylated also in the reaction of chymotrypsin and NPA. The resulting monoacetyl chymotrypsin can be isolated[20] and is remarkably stable in acid media, while rapidly hydrolysing between *p*H 7 and 8 (refs. 4 and 5). A variety of acylchymotrypsins was reported to be obtainable by reaction of the enzyme with stoichiometric amounts of acid anhydrides or acid chlorides[4]. All these derivatives are also stable in acid media and hydrolyse in neutral to slightly alkaline media. The dependence of hydrolysis on *p*H (as well as of acetylation of NPA) seems to involve catalysis by a neighbouring imidazole group. Direct spectrophotometric evidence for the intermediary formation of an N-acetyl imidazolyl derivative during the process of deacetylation was presented[16]. Detailed mechanisms for the reaction between the enzyme and NPA were postulated by several authors[19,21,22]. The same mechanism was extended also to the action of chymotrypsin on its normal peptidic substrates, though the evidence for it is largely indirect. For example, the *p*H dependence of hydrolysis of acetyl L-phenylalanine ethyl ester also implicates catalysis by an imidazole group[23]. This mechanism considers proteolytic enzymes as having a transacylating function, with water as the ultimate acyl group acceptor. It is most unfortunate that there is as yet no direct evidence for the actual formation of the acylated intermediates during peptidic hydrolysis.

In its reaction with NPA, trypsin behaves essentially similarly to chymotrypsin[4]. Exact comparison of the kinetic parameters and the *pK* values of the catalytic groups were first presented by Stewart and Ouellet[24]. A stable, inactive monoacetyl trypsin could, however, not be obtained by the authors following the technique used for chymotrypsin. Nevertheless, the rate of hydrolysis of the acetyltrypsin formed increases with *p*H at around neutrality, as shown by stopped-flow technique[24], and was easily confirmed by us.

In striking contrast with the foregoing results are the present results with acetic anhydride. The inactive acetyltrypsin obtained is unstable in acid media, but quite stable in neutral to slightly basic buffers. Similar data were obtained for a variety of other anhydrides[11]. The instability in acid media explains why no inactivation of trypsin could be observed, following the procedure[4] for the reaction of chymotrypsin with acetic anhydride (*p*H 5).

The acetylated group is presumably part of the active centre and is either an aliphatic or an aromatic hydroxyl group. The latter assumption is supported by its reactivity with hydroxylamine, which is intermediate to that of ethyl acetate and those of NPA or phenylacetate. Other groups seem excluded, as Uraki *et al.*[25] have shown that N-acetyl groups are resistant to hydrolysis even under stringent conditions, while the spectrophotometric data presented here preclude the acetylation of imidazole. The difference in *p*H dependence of spontaneous hydrolysis of the acetyltrypsin formed with NPA or with acetic anhydride would imply that two different hydroxyl groups are acetylated and are present in the active site of trypsin. The stability in alkaline media would apparently preclude the possibility that this latter acetyltrypsin is an intermediary in the course of normal enzyme action. An alternative possibility is that the difference in behaviour is due to the simultaneous acetylation of other groups as well, aside from the critical hydroxyl group, by the action of acetic anhydride, but not by the action of NPA. The group acetylated could be a primary amino-group, catalytically active in the deacetylation (and presumably also acetylation) of acetyltrypsin formed by the reaction of trypsin with NPA. This would be in agreement with the results of Stewart and Ouellet[24], who have shown that the hydrolysis of NPA can be catalysed by a combination of a free amino-group and an aliphatic hydroxyl group, namely, lysine methyl ester and *iso*propyl alcohol. The acetylation of the amino-group by the acetic anhydride would eliminate its catalytic role, accounting for the comparative stability of the inactive acetyltrypsin in neutral to alkaline media, while the hydrolysis in acid media may be due to simple acid catalysis. This would explain the difference in the behaviour from chymotrypsin, where an imidazole group has the same catalytic function.

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MECHANISM OF COLLAGEN SYNTHESIS

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EXPERIMENTAL evidence[1-3] indicates that the mechanism underlying the biosynthesis of fibrous proteins, such as collagen or silk fibroin, differs from that operating in the case of globular proteins[4-7]. In this article a mechanism is proposed for the biosynthesis of collagen which could account for the experimental data available at present. This mechanism is based on the notion that collagen is synthesized by an enlargement of the pre-existing collagen fibre by means of the addition of amino-acids or peptide residues and would be analogous to that proposed for hepatic glycogen[8]. The proposed synthetic process is unlike that visualized for globular proteins, which are thought to be synthesized by one continuous process[4-7]. The *a priori* assumption seems reasonable that a long fibrous molecule which is considerably longer than the ribonucleic acid templates[9-11] is probably not synthesized in a single operation but rather by a step-wise addition of simple building blocks. The latter view is supported by direct evidence since it has been observed in this laboratory that the distribution of an isotope among identical amino-acid residues is non-uniform[1,2] after the injection of amino-acids labelled with carbon-14. A similar non-uniform labelling pattern has been found in the case of silk fibroin[12]. It is therefore postulated that discrete regions near the termini of the collagen molecule participate actively in the metabolism of this protein whereas regions distant from these termini are metabolically inert.

By varying the ionic strength or *p*H of the medium, fractions of collagen can be prepared differing from native collagen with respect to their isotope concentration and rate of metabolic replacement[13-18]. Although these fractions have generally been assumed to represent distinct molecular entities within the collagen group, a more plausible, alternative view would be that these fractions originate from the same molecular species of native collagen by a process of intramolecular cleavage. The following evidence may be adduced for the common origin of these fractions: (*a*) all collagen fractions derived from the same organs have almost identical amino-acid composition[19]; (*b*) when collagen is solubilized, the resulting fractions have similar molecular weights[2]; and (*c*) on sub-fractionation of native collagen, C- and N-terminal amino-acids become discernible in the resulting products[21-23].

The latter observation suggests that intramolecular cleavage occurs during the fractionation procedure. Certain peptide bonds, particularly those involving aspartyl residues[24], are probably ruptured preferentially. Specifically, it is postulated that peptides situated near the termini of a polypeptide chain of native collagen become detached under mild experimental conditions and give rise to neutral-salt-soluble collagen. Only when native collagen is treated more vigorously will amino-acid sequences located at a greater distance from the termini of the fibre become severed to form citrate-soluble collagen plus a residual core of insoluble collagen.

It follows from the preceding considerations that differences in the metabolic turnover of various fractions of collagen should be determined by differences in the distance of the point of intramolecular cleavage from the metabolically active terminus of the fibre. Such a view would offer a reasonable explanation for the higher level of isotope incorporation and the more rapid replacement of the fraction of neutral-salt-soluble collagen as compared with other fractions, such as insoluble collagen, since the former fraction is thought to have originated from peptide sequences vicinal to the termini of the fibre, whereas the latter fraction represents the metabolically inert core of the molecule.

In view of such apparent differences in the metabolism of various collagen fractions, speculation as to size and metabolic behaviour of the fundamental repeating unit of collagen seems pertinent. On the basis of the amino-acid composition and the nature of the peptides isolated by various investigators from partial hydrolysates of collagen, it seems unlikely that molecular enlargement takes place by means of the addition of single amino-acid residues to the termini of the fibre in a random fashion. It appears more probable that a polypeptide, pre-formed on a template, is added to the collagen fibre, thereby enlarging the molecule. The size of such a polypeptide building block can be delimited. The smallest possible size can be computed from the amino-acid composition whereas the largest possible size can be estimated on the basis of metabolic data pertaining to the incorporation of amino-acids labelled with carbon-14 (refs. 1 and 2).

The residues of glycine, proline, hydroxyproline, alanine, arginine, and glutamic acid recur with unusual frequency in the peptide chains of collagen and constitute 73 moles per cent of all amino-acid residues of this protein. Aspartyl, seryl and lysyl plus hydroxylysyl residues occur in concentration of 3–6 moles per cent, whereas valyl, *iso*leucyl, phenylalanyl, methionyl and threonyl residues occur in concentrations of 1–3 moles per cent. Histidyl and tyrosyl residues occur least frequently, namely, in concentrations of 0·7–0·9 mole per cent[25]. When computed on the basis of the amino-acid occurring least frequently, the smallest building block should contain about 140 residues and should have a molecular weight of about 12,000. Alternatively, it is conceivable that under the following two conditions the amino-acid residues recurring least frequently do not determine molecular size: (*a*) residues having a low frequency of recurrence appear only as constituents of branching chains which are formed later than the peptide chain, or (*b*) these residues form the link between two building blocks. Although the recent isolation of the tripeptide involving the ε-amino group of lysine[26] suggests the existence of such side chains, condition (*a*) appears unlikely since both amino-acid residues would have to occur in side-chains. If condition (*b*) were to prevail and the building blocks had polar and non-polar termini, these building blocks could be linked through histidyl or tyrosyl residues in two ways: (*a*) in a complementary way, that is, non-polar.polar–polar.non-polar, or (*b*) in an alternating fashion, that is, polar.non-polar–polar.non-polar. The complementary arrangement would have the advantage of accommodating readily both histidyl and tyrosyl residues so that these residues might serve in the capacity of bidentate links at the polar and non-polar termini, respectively. If these assumptions are valid, the lower limit for the size of the building block would be approximately 70 amino-acid residues and the minimal molecular weight about 6,000. Such a view is compatible with data on the composition of several polypeptides containing 20–80 amino-acid residues isolated by Grassmann *et al.*[27] as well as with the findings of Schroeder *et al.*[28], Heyns *et al.*[29] and Kroner *et al.*[30].

The upper limit for the size of the building block can be set only in terms of the ratio of the size of the building block to that of the collagen molecule. Since identical amino-acid residues in the collagen molecule are labelled non-uniformly, a ratio of about 1 : 3 for the highest to the lowest specific activity of such amino-acid residues may be considered a measure of the relative size of the fundamental building block.

The ratio of the size of the building block to that of the collagen molecule can also be computed from the ratio of the specific activities or of the turnover times of urinary 'free' and 'bound' hydroxyproline[3]. The former has a turnover time of about 0·5 day and the latter a turnover time of 15 days. The specific activity extrapolated to time zero after injection is about 50 times higher than that of bound hydroxyproline[3]. If one postulates that 'free' hydroxyproline is derived from the building blocks and 'bound' hydroxyproline from native collagen, the size of the building block may be estimated at 1/20 of the size of native collagen and would have a molecular weight of less than 3×10^5 as compared with an average molecular weight of 5×10^6 assumed for native collagen. The high specific activity in urinary 'free' hydroxyproline may also be a consequence of the unique process by which proline is hydroxylated when this amino-acid is part of a precollagenous peptide or related substances. One may postulate that the hydroxylation of proline occurs only at specific sites of the template at a time when constituents of the peptide chain are still incompletely assembled. Hydroxyproline may be assumed to have a low affinity for the template which facilitates the synthesis of the building blocks since hydroxyproline is known not to be incorporated into the nascent peptide chain of collagen and can arise only from bound proline[31–33]. Consequently, it is possible that certain hydroxyproline residues are eliminated from the template and constitute in part the source of 'free' hydroxyproline.

It is of interest to point out that an inverse relationship appears to exist in various collagen fractions between metabolic activity and carbohydrate content[19–24]. This fact may account for the differences between collagen fractions with regard to their respective solubility characteristics[25]. In spite of the low carbohydrate content of 1 per cent or less[36], the polysaccharide material may be of considerable physiological importance. One may speculate that the metabolically active terminal sites of collagen are capable of reacting with either polysaccharides or the peptide building blocks. The reaction with polysaccharides may be considered irreversible in nature and to proceed at an appreciably slower rate than the reaction with a building block, thus preventing further additions of building blocks at the sites involved. On the other hand, the reaction with building blocks is reversible and leads to enlargement or to branching of the collagen molecule. The simultaneous operation of these two reactions at the metabolically active sites of collagen would tend to restrict the process of branching and would, furthermore, provide a means of controlling the length of the peptide chain of collagen. Such a concept might be applied fruitfully to the study of metabolic inertness in relation to the process of ageing. In conclusion, it may be stated that the mechanism proposed here for the biosynthesis of collagen, although not entirely proved, suggests new avenues of approach to the study of connective tissue proteins.

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COMPARATIVE EFFECTS OF LIVER AND TUMOUR RIBONUCLEIC ACIDS ON THE NORMAL LIVER AND THE NOVIKOFF HEPATOMA CELLS OF THE RAT

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THE Novikoff hepatoma of the rat seemed to be an appropriate model to test some of the hypotheses derived from experiments with human tumour ribonucleic acid (RNA)[1-6]. It is easy to transplant, and it offers the opportunity to compare experiments with the normal counterpart organ, the liver.

RNA from normal liver and spleen and kidney from Novikoff tumour of adult (250-gm.) female Sprague–Dawley–Holtzman rats was extracted by both phenol and fluorocarbon methods[2-4,6]. 50 gm. of normal or hepatoma tissue were thrown on dry ice and extracted immediately or preserved at −20° C. for a few days. The RNA preparation was always used on the same day of preparation (less than 2 hr. at 2° C.). The ultra-violet spectra of liver RNA and hepatoma RNA show the expected difference due to the reported richness of the latter in guanine[7] (Fig. 1). The hyperchromatic effect of both RNAse and temperature indicated near-native, undenatured state.

The Novikoff tumour on the fifth day of transplantation was collected and broken in an L. Blessig manual homogenizer in phosphate-buffered saline (5 gm. of tissue in 25 ml. of salt solution). The homogenate was filtered through two layers of gauze and the cells washed four times in the RNA solvent (0·06 *M* disodium versenate, 0·06 *M* phosphate buffer *p*H 7·0, 0·12 *M* sodium chloride).

The cells are counted and then re-suspended in the RNA solution at a ratio of 2,500 μgm. RNA/10^6 cells. The suspension is left in a large bottle lying on the side to prevent cell accumulation, at 2° C. for 15 hr.

It is known that when 90 mgm. of tumour tissue are injected intraperitoneally 92 per cent of the animals have 5–8 gm. omental tumours in 5–8 days[8]. Our routine passages confirmed these data.

In one experiment 40 animals received hepatoma cells four times washed with buffered versenate–saline, and left for 15 hr. at 2° C. (group 1). Another 40 animals received hepatoma cells incubated with normal rat spleen RNA (group 2), 40 hepatoma cells incubated with kidney RNA (group 3), 40 with hepatoma cells incubated with normal rat liver RNA (group 4) and another 40 with hepatoma cells incubated with hepatoma RNA (group 5). Table 1 summarizes the results of this experiment.

Tumours in groups 4 and 5 were different from routine tumours, such as in groups 1, 2 and 3. All were considered to be modified tumours; the modifications in group 4, however, were in opposite directions from group 5.

Tumours in group 4, derived from hepatoma cells treated by normal rat liver RNA, were small (2 mm. in size) slightly hæmorrhagic nodules scattered in the mesentery, and never formed a solid omental tumour. Histologically, they showed a more orderly cordonal organization with the tendency to radiate around a vein (Fig. 3). The nucleo-cytoplasmic and nucleolo-nuclear ratios were not as markedly increased as in the routine tumour, and the cells contained glycogen in a polarized distribution, resembling normal liver. The ascitic fluid was bloody, but also contained bile elements. Mitoses were infrequent. The morphochemical characteristics, the slow growth, the low percentage of takes and the

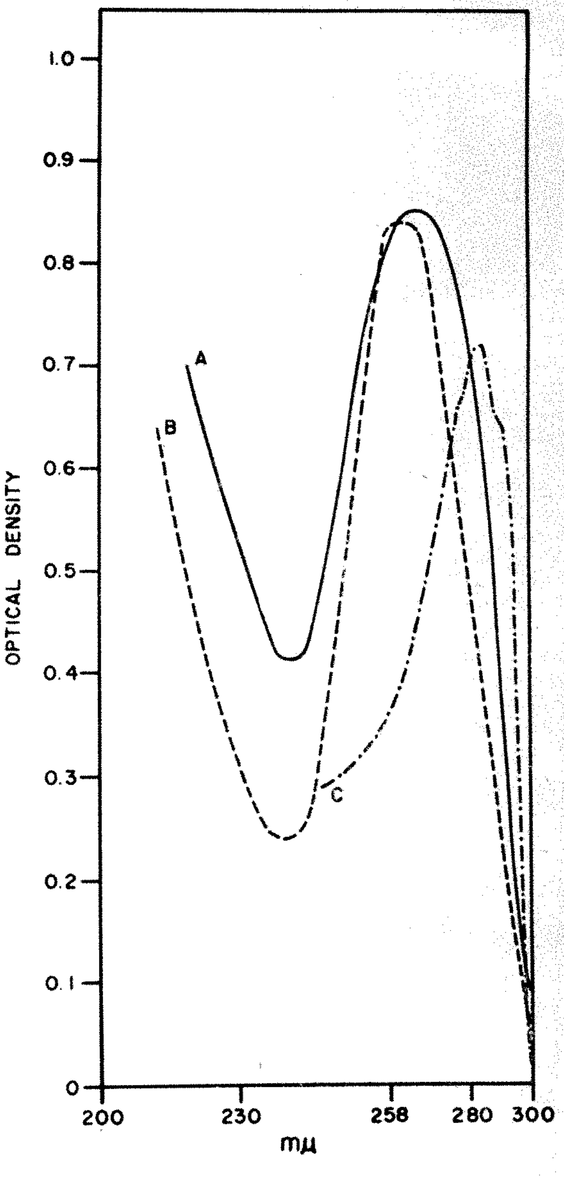

Fig. 1. Ultra-violet spectra of 10^{-3} dilution of phenol in 1/15 *M* phosphate buffer in 0·12 *M* sodium chloride, *p*H 7 at 20° C. (*C*); phenol-extracted RNA from Novikoff hepatoma (*B*); and RNA from Novikoff hepatoma modified by normal rat liver RNA (*A*)

Table 1. Effects of Different RNA Preparations on Novikoff Tumour in Intraperitoneal Grafting

Group	RNA source	Percentage of tumours	Mean time of death (days)	Percentage of regressions
1	—	95	6	0
2	Rat spleen	94	6	0
3	Rat kidney	95	6	0
4	Rat liver	15*	12	85
5	Hepatoma	100	6	5

* Thirty-four animals still alive without tumours 95 days after inoculation. These animals were resistant to further tumour transplantations.

practical lack of lethality indicate a certain degree of re-differentiation of the original wild malignant tumour cells by the normal RNA preparation. That this action is specific to liver RNA is indicated by the fact that rat spleen and rat kidney RNA both failed to modify the tumour. These tumours were passed intraperitoneally in the usual way and did not produce the usual picture of the large malignant hæmorrhagic omental tumour in six days.

In Niu's experiments[9] Ehrlich ascites tumour cells of the mouse produced serum albumin and lost anaplastic features after treatment with liver RNA (from a mouse and a calf). It would be of interest to know whether or not they had regained malignant virulence on re-transplantation.

Since calf liver RNA in Niu's experiments was able to perform a liver-oriented differentiation (serum albumin secretion) by tumour cells, we decided to test the influence of heterologous liver RNA (rabbit) on the rat hepatoma. The following experiment was performed: eighteen rats were inoculated intraperitoneally with hepatoma cells treated by rabbit liver RNA (group 1). Six rats were inoculated with hepatoma cells treated by rabbit spleen RNA (group 2). Six rats were inoculated with hepatoma cells from the same batch used in group 1, washed in buffered saline and left for 15 hr. at 2° C.

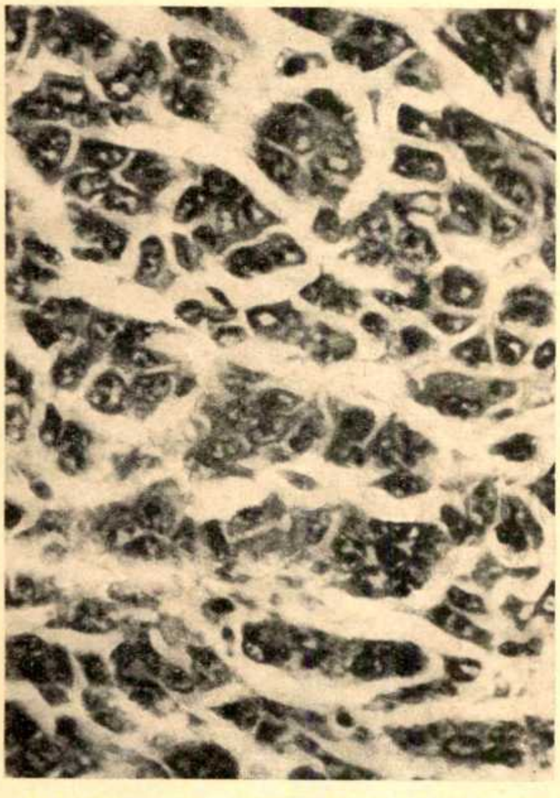

Fig. 2. Omental tumour, Novikoff hepatoma. (Histoid pattern × 275)

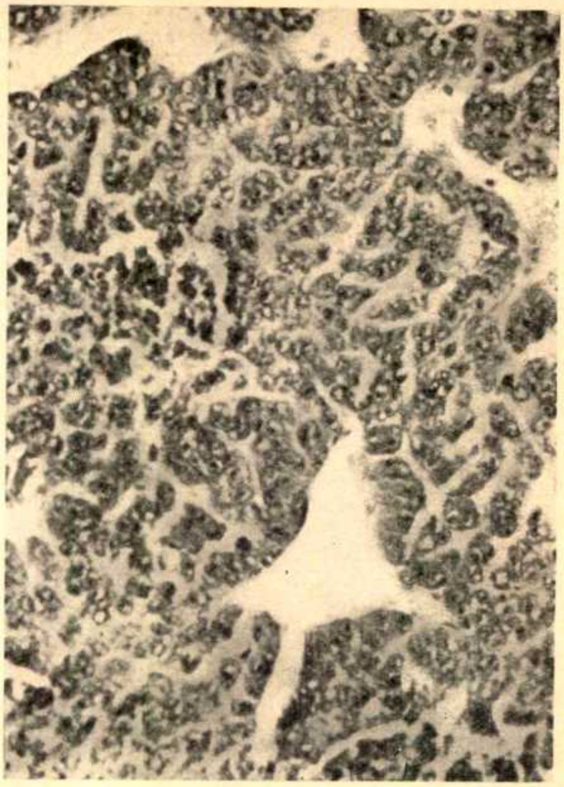

Fig. 3. Mesenteric nodule from transplantation of hepatoma cells previously treated by RNA from normal rat or rabbit liver. (Organoid pattern × 175)

An important determinant in the outcome of these experiments of tumour modification by normal RNA is the proportion of number of cells per mole of RNA. Any cell that will not incorporate enough or no RNA at all could be responsible for the restoration of the wild tumour. The exact conditions were not established in these experiments. An excess of RNA was always used to circumvent these pitfalls.

The following were the results on the sixth day after the transplantations:

Group 1. No large tumours visible. No anæmia or signs of hæmorrhage; less activity. Two animals were sampled. One had a few nodules of 1–2 mm. size in the omentum which on histological examination proved to be similar to the modified organoid type of tumours obtained with normal rat liver RNA.

Group 2. All animals had large tumours anæmic and weak; two had died on the fifth day.

Group 3. Three animals died on the sixth day with large omental tumours; the three remaining all had large tumours.

This experiment and Niu's seem to indicate that RNA from the different organs has morphogenetic-differentiating and enzyme-forming capacities that transcend species barriers and are common to homonymous organs of different species. This phenomenon is parallel to the similarity of endocrine secretions in heterologous systems and may be a fortunate event since it may allow use of specific

Table 2. EFFECTS OF DIFFERENT RNA PREPARATIONS ON NORMAL RAT LIVER CELLS

Group	Source of RNA	Percentage of tumours	Mean time of appearance (days)	Percentage of regression
1	—	0	—	
2	Liver	0*	—	
3	Hepatoma	20	15	5

* Small fibrotic nodules were sometimes found on the surface of the liver.

Table 3. EFFECT OF HEPATOMA RNA ON THE RAT

Group	Route	Percentage of tumours	Mean time of appearance (days)	Percentage of regression
1	Adult intraperitoneal	30	15	0
2	Prenatal* intravenous	10	25	40
3	Neonatal subcutaneous	5	27	40

* Results refer to offspring.

ribonucleic acids of animal origin in situations of enzyme deficiencies in man.

Supravital stainings such as acridine orange of tumour cells after 15-hr. incubation with liver RNA indicate viability of the cells after such treatment. Damage is also ruled out by the lack of effect of similar RNA preparations from other organs and from the tumour itself. Treatment of hepatoma cells with hepatoma RNA resulted in a wilder modification of the tumour. Within six days the tumour showed not only the usual large omental mass but also that this would extend to the liver, diaphragm, abdominal walls, whole peritoneal cavity, ovaries, intestines and into the pleural and pericardial cavities with a peculiar involvement of the thymus and the mediastinal and axillary lymph nodes. The thoracic involvement was noticed as extreme dyspnœa and cyanosis. In spite of all its wild features, this modified tumour regressed in a larger percentage than the unmodified variety.

In another experiment 50 animals received normal rat liver cells simply washed (group 1), treated by liver RNA (group 2) and treated by hepatoma RNA (group 3). The results are shown in Table 2. Tumours in group 3 consisted of slightly hæmorrhagic nodules deep-seated in the hilum and septa of the liver with scattered mural implantations. To test whether the results of the last experiments were due to a modification of the liver cells or to a direct effect of the tumour RNA this was injected intraperitoneally into adult rats, intravenously prenatal and subcutaneously neonatal. Controls consisted of normal liver RNA and buffered versenate saline (Table 3).

Of the controls injected with normal rat liver RNA, the prenatal and neonatal showed marked retardation of growth and stunting, but no tumours in the same period that the animals exposed to tumour RNA developed tumours. Eventually, the controls resumed normal growth and development while the experimental ones died of abdominal hæmorrhage.

These tumours extended throughout the peritoneal cavity, but serial sectioning of the liver and of the pancreas shows areas of possible primary tumour not found usually in the commonly transplanted hepatoma. Novikoff reports that the pancreas can be infiltrated by the tumour[8]. This causes the peritoneal implantations metastases, such as in the original Novikoff primary hepatoma. Interesting is the pancreas lesion, since liver and pancreas are two embryologically related organs. No thymus, lymph node or spleen involvement nor tumours of other localities were seen in these young. The possibility of malignant changes in liver cells induced by hepatoma RNA cannot be excluded. Niu[9] reports the formation of local tumours when normal mouse kidney cells were treated by RNA from ascites tumour cells. However, no attempt to produce this with RNA alone has been reported.

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BLOOD NUCLEASES AND INFECTIOUS VIRAL NUCLEIC ACIDS

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IT has been shown that animal viral nucleic acids can infect tissues[1-14] and that this is not blocked by antiserum against the intact virus[3,9,11]. Since nucleases in very low concentration destroy biologically active nucleic acids[15,16], an investigation of the deoxyribonuclease (DNAase)- and ribonuclease (RNAase)-levels in human blood was undertaken to decide their possible role against infectious deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

In the course of this investigation we encountered the liberation of both nucleases and their inhibitors from the cellular elements of blood as well as the formation of a DNAase inhibitor in serum. Analyses of these materials are included because they suggest explanations for changes in nuclease-levels that are otherwise difficult to understand.

Materials and Methods

Only an outline of procedures is given here. Detailed reports will appear elsewhere. Unless otherwise stated, all enzyme or inhibitor assays were performed at 37° C.

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DNAase assay. The substrate of this enzyme was highly purified *Hemophilus influenzae* transforming DNA which can be assayed at concentrations as low as 10^{-6} μgm./ml. A concentrated solution of this DNA which confers streptomycin resistance on a susceptible strain of this organism was added to blood, blood products, or a solution of crystalline pancreatic DNAase (Worthington Biochemical Corporation, Freehold, New Jersey) dissolved in blood electrolyte solution at *p*H 7·4. The concentration of the transforming DNA in the digestion mixture was 1 μgm./ml. The blood electrolyte solution was made up following data by Bland[17] to which was added 10 μgm./ml. of crystalline bovine serum albumin (B.E.S.A.). Samples were withdrawn at intervals and assayed for residual transforming activity by the usual procedure[18]. The slope of a curve through such points was then used to get from a standard curve of an equivalent action by known concentrations of a crystalline preparation dissolved in B.E.S.A.

DNAase inhibitor (*DI*). DNAase inhibitor was determined by its action against a standard pancreatic DNAase solution analysed in the same manner. A dilution of the inhibitor which reduced the rate of destruction of transforming activity by 0·001 μgm./ml. crystalline DNAase in B.E.S.A. to one-half the control value was considered as containing 1 unit of DI.

RNAase assay. A concentrated solution of yeast RNA (Schwarz and Co., Mt. Vernon, New York) at *p*H 7·4 was added to blood, blood products, or solutions of crystalline pancreatic RNAase (Worthington Biochemical Corporation) in B.E.S.A., bringing the final concentration to 10 μgm./ml. At minute intervals samples were removed and the extent of digestion measured spectrophotometrically at 260 mμ on a filtrate after chilled perchloric acid precipitation of the undigested RNA. Blood-levels of RNAase were evaluated in terms of equivalent action by known concentrations of crystalline pancreatic RNAase (Worthington Biochemical Corporation).

RNAase inhibitor (*RI*) *assay.* RNAase inhibitor was assayed by its action against 0·1 μgm./ml. of crystalline RNAase. As with DNAase, the dilution of inhibitor which reduced the action of the standard enzyme solution to half its value was thought to contain 1 RI unit.

Blood, plasma and serum. Preliminary experiments, even in the absence of anticoagulants, gave variable results with whole blood, whereas consistent and otherwise similar results were obtained with the plasma. Assays were therefore made on plasma in the absence of anticoagulants, since the latter affect the DNAase. Samples were drawn freshly through silicone-coated ('Siliclad', Clay-Adams, Incorporated, 141 E. 25th St., New York 10, N.Y.) needles and syringes and introduced into chilled coated tubes; spun at 8,000 revolutions/min. for 5 min. and the nuclease-levels of the plasma evaluated immediately. Serum was prepared by allowing freshly drawn blood to incubate for 1 hr. at 37° C. after which the serum was drawn off, centrifuged when necessary and chilled or analysed immediately.

White blood cells (*WBC*). Blood drawn as above was centrifuged horizontally at 2,000 revolutions/min. for 10 min. The plasma was almost completely removed and used for the isolation of platelets. The WBC of the packed cell portion on standing 5–10 min. became mixed with the residual plasma. This was removed and diluted with B.E.S.A., the cells were centrifuged and, after a second washing, they were re-suspended in B.E.S.A.

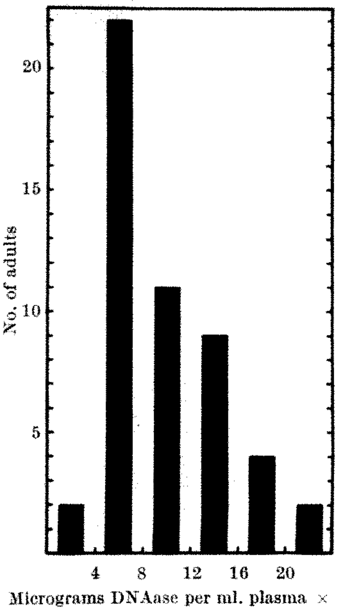

Fig. 1. DNAase-levels per ml. plasma of 50 normal adults

Disruption of cells. Each cell-type was suspended in a volume of B.E.S.A. to bring it to the concentration-level found in the blood. Disruption was obtained in 'Lusteroid' tubes placed in a 10-kilocycle Raytheon sonic oscillator. At full power (250 watts), disruption was essentially complete in 2·5 min. for platelets; 2 min. for WBC, and 10 min. for red blood cells (RBC).

Experimental Results

Nuclease-levels in blood of adults. Fig. 1 shows levels of DNAase in the plasma of fifty healthy adults (25 females and 25 males). Representatives of the coloured races are included. Evidence (not shown) that the inactivation of transforming DNA is probably due to the DNAase in blood is suggested by (*a*) a quantitative correlation between destructive rate and concentration of serum, and (*b*) an independent viscosimetric analysis of serum DNAase-level. Plasma was used regularly in place of whole blood with comparable results, considering the volume difference. From Fig. 1 it appears that the range of DNAase-level in plasma of human adults is 0·0003–0·002 μgm./ml. with an average of about 0·001 μgm./ml. and half these values per ml. of whole blood. The DNAase is Type 1 (ref. 19) since it has a digestion maximum near *p*H 7.

As noted earlier[20], blood DNAase depends on divalent cations for activity; hence it is inhibited by citrate, ethylenediaminetetraacetic acid (EDTA) and other chelating agents. In the present work heparin as an anticoagulant was avoided because as little as 40 μgm./ml. inhibited plasma DNAase, yet had no apparent effect on the pancreatic depolymerase. The literature is confusing in this regard since reports indicate that heparin inhibits pancreatic DNAase[21], does not inhibit the serum enzyme[22], and that it inhibits both[23]. Some features of the system not yet understood must be critical to this reaction.

The results in Fig. 2 suggest that the level of RNAase in the blood is equivalent to approximately 0·10 ±

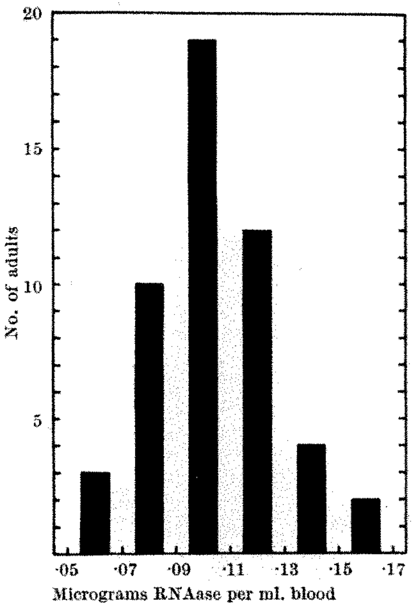

Fig. 2. DNAase-levels per ml. blood of 50 normal adults

0·05 μgm./ml. of crystalline pancreatic enzyme. Under the present conditions of study, the level of RNAase was not affected by clotting or manipulation. This result is somewhat lower than that reported earlier[2,26,27] for serum, even when correcting for the difference in volume.

Nucleases and their inhibitors in the formed elements. The DNAase activity of whole blood incubated at 37° C. decreased with time, suggesting the formation or liberation of an inhibitor. This, plus the observation that serum DNAase-levels were 2–6 times higher than the corresponding plasma-level, led to an analysis of isolated washed cellular components of blood sonically disrupted in B.E.S.A. The results are summarized in Table 1.

A sonicate of platelets contained a DNAase which could be activated by Mg^{++}. It was equivalent to 1×10^{-11} μgm. of crystalline pancreatic DNAase per platelet. These samples of blood with about 5×10^{8} platelets per ml. contain 5×10^{-3} μgm./ml. DNAase, or ten times as much as found free in whole blood, but nearly the same concentration as found in serum from clotted blood. It seems reasonable, though not proved, that the higher serum DNAase (see Table 1) arises from disruption of platelets during clotting, for the DNAase-level of serum from clotted cell-free plasma is not higher. It is somewhat lower due, perhaps, to adsorption of the enzyme to the clot.

Table 1. Average Levels of Nucleases and their Inhibitors in Blood and Certain Blood Fractions

System	DNAase μgm./ml.	RNAase μgm./ml.	DNAase inhibitor units	RNAase inhibitor
Whole blood, fresh	0·0005 ± 0·0002	0·1 ± 0·05		
Plasma, fresh	0·001 ± 0·0004	0·2 ± 0·1		
Serum, fresh (from clotted whole blood)	0·006	0·2		
Serum (clotted cell-free plasma)	0·0003			
Platelets (blood concentration)	0·005			
WBC (blood concentration)	0·0	0·1	300	
RBC (blood concentration)			350	10
Serum heated at 56° C. 0·25 hr.		0·2	2	
Serum at 37° C. 15 hr.			3	
Serum + 0·01 *M* Mg^{++} 1 hr.		0·2	100	

The presence in white blood cells of a powerful DNAase inhibitor had been known[24,25]. In the present system 1 : 300 dilution of the sonically disrupted WBC at a concentration found in whole blood, reduced the DNAase activity of a standard enzyme preparation to one-half. So, by definition, the WBC at blood-level contains 300 DI. Much of this inhibitor is released into the medium on incubation at 37° C., which may be responsible for the observed variations in enzyme-levels.

Similar sonicates of WBC contain RNAase, and it has been suggested that white blood cells are the source of serum RNAase[26].

Inhibitors of both nucleases were found in the solutions of disintegrated, washed red blood cells. Expressed in units defined in the section on Methods there were 350 DI and 10 RI per ml. of RBC at the blood-level concentration.

Formation of DNAase inhibitor in serum. As noted earlier, the level of DNAase in fresh serum is several times higher than that in plasma, yet, under certain conditions, this activity is lost and it seems that an inhibitor is formed in the serum. Previous workers[20,22] had noted a loss of DNAase on heating but had offered no explanation. Since the heated serum will inhibit added pancreatic DNAase, an inhibitor for this enzyme would appear to have been formed. The conditions favouring inhibitor formation are (*a*) incubation overnight at 37° C., (*b*) incubation at 37° C. for 1–2 hr. after addition of 0·01 *M* Mg^{++}, or (*c*) heating at 56° C. for 15 min. The levels of inhibitor formed are shown in Table 1. The loss of enzymic function was not due to a loss of Mg^{++} activator, for addition of the latter brought about no restoration of activity. It is difficult to account for the difference between the present results showing formation of inhibitor at 37° C. and the reported[28] destruction of pancreatic DNAase inhibitor at 35° C.

Discussion

It seems reasonable to suppose that tissues undergoing a viral infection contain free viral nucleic acid as well as complete virus, and both these would be liberated at some stage of the infection. While complete virus can be rendered non-infectious by antibodies, the infectivity of the nucleic acid is apparently not reduced by this mechanism[3]. One wonders if the nucleic acid phase continues to spread the infection even if less effectively, or if there is another physiological defence against this phase as well.

The present work, which is confined to blood, shows that healthy human adults have nucleases which destroy biologically active nucleic acids of both types, and the question comes down to one of competing rates, that is, the rate of enzymic destruction versus the rate of infection, and the decision will be determined by the concentrations of nuclease, viral nucleic acid and the number of susceptible cells.

Work on the infectivity of viral RNA shows that normal serum destroys infectivity[1,3,6], and this destructive power is attributed to the RNAase[3]. The present results support this conclusion, for RNAase activity is found in serum.

The rate of destruction of transforming activity by pancreatic DNAase in B.E.S.A. is about 170 logarithmic units per minute per microgram of crystalline enzyme. From results presented here, there is only 5 × 10^{-4} µgm. of DNAase per ml. of blood in the average healthy human being ; hence, destruction will go on at the rate of about 1 logarithmic unit per 12 min., that is, 90 per cent destruction in 12 min. There is too little information on the minimum time necessary for infection in human viral diseases, especially for the naked nucleic acid phase, to draw a firm conclusion about blood DNAase as an effective defence against infectious DNA. But general considerations from work on tissue cultures[3,24] and bacterial transformations[30-32] suggest that infection of susceptible tissues by a viral nucleic acid takes place in a few minutes or less.

Schramm and Gierer[16] reported the destruction of infectivity of tobacco mosaic virus RNA by crystalline pancreatic RNAase at 8° C. Applying the usual temperature coefficient for enzyme-catalysed reactions (2 per 10 deg. C.) leads to a value for the rate of destruction at 37° C. of about 300 logarithmis units per min. per microgram of crystalline RNAase. A blood level of 0·1 µgm./ml. of RNAase would then be expected to destroy the infectivity of free RNA at the rate of 30 logarithmic units per min. per ml., or 0·5 logarithmic units per sec. Whether this is rapid enough to prevent infection is a matter for conjecture, but it is about one hundred times faster than the rate at which DNAase destroys DNA. In this connexion it should be recalled that infections are produced in animals with viral nucleic acid. Thus both RNA[4,6,8] and DNA[13] have produced infections, and in at least one case the route of infection was significant[6]. It is possible, however, that these laboratory infections developed only because relatively high levels of nucleic acid were inoculated and that the nucleases and other counteracting forces eliminate the low levels of nucleic acid that appear during the natural disease.

The experience of many virologists in completely neutralizing sera from those human beings who are ill with viral infections cannot be used as an argument against the existence of a nucleic acid phase, for the time consumed in preparing the sera and the increased DNAase-levels of serum could well militate against detection of any nucleic acid originally present in the donor's blood.

The presence of DNAase in platelets and its inhibitor in white blood cell, red blood cell and serum probably explains the wide variations in reports on blood or serum concentrations of this enzyme[20,22,34]. The RNAase in white blood cells and its inhibitor in red blood cells are not sufficient to lead to any appreciable variation in RNAase-levels in ordinary circumstances. Perhaps the wide fluctuations in DNAase-levels and its inhibitor, compared with the relatively constant level of RNAase, are due to the extremely low normal level of DNAase. The release of quite small quantities of either the enzyme or its inhibitor produces large changes in the blood level of this enzyme.

Some variations in the levels of these enzymes in the blood of sick individuals will be the subject of another communication. A few analyses of blood from individuals suffering from serum hepatitis showed no depressed levels of blood nucleases. In this anomalous disease, which prompted the present work, the long incubation and carrier periods[35] suggested a non-antigenic agent and hence, possibly, viral nucleic acid. These preliminary analyses, even if substantiated, do not exclude the possibility of a free viral nucleic acid from being important in this disease; they merely show no alteration in the nuclease-level.

An assumption, not yet tested, is involved in this work and it deserves to be mentioned. Implicit in the quantitative analysis is the assumption that the viral nucleic acids would exhibit the same response to nucleases as the arbitrarily chosen substrates. There are few data for comparison, except for experiments in which poliomyelitis RNA[3] was used. In this case, it was found that monkey serum contains RNAase equivalent to about 0·6–5 µgm./ml. of crystalline pancreatic RNAase, and species differences can be considerable[36].

Finally, it may be worth noting that the usual means of preserving blood or blood products, especially the use of chelating agents such as citrate as anticoagulant, will preserve viral DNA in an infective state by tying up the divalent cations needed for DNAase action, and methods such as heating to 60–65° C., designed to sterilize certain blood fractions, do not destroy the biological properties of DNA[37].

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LETTERS TO THE EDITORS

PHYSICS

The Bertele Arc

THE electron emission in the cathode spot of arcs has remained an unexplained phenomenon since K. T. Compton[1] first directed attention to it. It appears that the arc discharge discovered by H. von Bertele[2] puts this paradox into an even more acute form.

In this discharge the cathode is a polished, water-cooled thimble of molybdenum, which first must be processed in a way which suggests the formation of a very thin layer of amalgam. The molybdenum thimble is kept in contact with a small pool of mercury. The cathode spot first runs freely about on the surface of the mercury pool, but it soon becomes attached to the boundary-line between molybdenum and mercury, on which it forms what appears as a luminous line. During further processing the cathode, spot gradually spreads over the molybdenum surface until it appears to cover it completely. In this operation a faintly luminous glow covers the cathode and to the eye the discharge has all the appearance of a glow discharge, while electrically it can scarcely be distinguished from an arc. The cathode drop is about 10 V., up to the highest currents which we have investigated, which were about 30 amp. on cathodes of about 1 $cm.^2$ area. The paradoxical feature of the phenomenon is the small drop in voltage, in combination with a current density which is many orders of magnitude higher than could be obtained in a glow discharge.

We have now further investigated the nature of this striking phenomenon by two lines of experiments: cinematographic observations and measurement of the current fluctuations. It was natural to suspect that the apparently steady and uniform discharge was simulated by smaller cathode-spots running about at high speed. This was in fact revealed by high-speed photographs, for which we are obliged to the Rectifier Department of the General Electric Co., Wembley. In the photographs shown, taken with an exposure of 1/15,000 sec. at intervals of 1/3,000 sec. of a cathode of about 1 $cm.^2$ area, at a current of 15 amp., the luminous glow filled at any time only about 1/10 of the cathode area. The glow has a complicated ramified appearance, and greatly changes its shape and position in intervals of 1/3,000 sec., but its area appears to remain approximately constant so long as the current is constant, and appears to increase proportionally to the current. In other words, judging from these photographs it appears that we have to do with a 'normal glow discharge', but with a current density about 150 $amp./cm.^2$ which is at least six orders higher than in the normal glow at such low pressures, though four orders lower than the current densities observed by Froome[3] in cathode spots on pool cathodes.

If this is the correct interpretation of the photographs, it appears that the cathode spot jumps rather than moves steadily from position to position, as its outlines remain remarkably sharp in photographs taken with an exposure of about 1/5 of the picture interval. It does not appear though that this violent motion is necessary for the existence of the spot. On the cylindrical mantle of the thimble one can observe in the photographs several small spots of which one has remained stationary for 1 millisecond, and in other photographs we have observed spots which were steady over periods ten times as long.

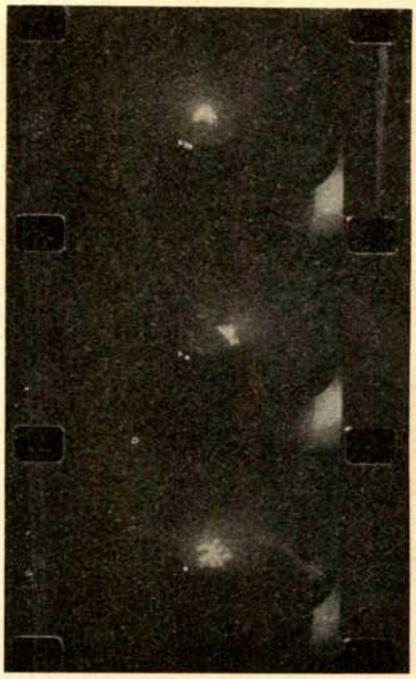

Fig. 1. Sequence of three photographs of the cathode spot in a Bertele discharge, at a picture-rate of 3,000/sec., exposure 1/15,000 sec. The cathode is a water-cooled molybdenum thimble with a blunt conical top and cylindrical shaft of 1 cm. diameter which is surrounded by an annular groove in an insulator, containing mercury during the forming, but empty during operation. Current, 15 amp.

There is, however, another possible interpretation. This is that the apparently large, diffuse spots are simulated by a number of much smaller very bright spots, running about at high speed in a restricted area, which changes from time to time. One could even assume that these spots are not essentially different from those on pool cathodes, because they are running about on a monatomic film of mercury which they rapidly evaporate. Such a hypothesis would not be absolutely contradicted by the small steady spots, because one could imagine the monatomic mercury film rapidly flowing into them from the periphery.

Unfortunately, we had no equipment available for repeating these observations at currents about 150–200 amp., at which the 'glow hypothesis' would predict that the whole surface of the cathode is permanently covered and the voltage drop would have to rise rapidly; nor could we repeat the experiment with a higher time resolution, which might have shown up the hypothetical smaller spots. We are inclined though to believe that the 'glow interpretation' is nearer to the truth, on the basis of our second line of experiments, on the fluctuation spectrum of the arc current.

We have measured the power spectrum of the current in the frequency range from a few hundred cycles to one megacycle. In the range up to 50 kilocycles the noise-power is flat; beyond this there is a rapid fall-off with a cut-off at about one megacycle. Quantitative analysis shows that the spectrum could be accounted for by the superposition of random bursts each with the discharge of only about one million electrons. The fall-off with frequency leads to the conclusion that these bursts have a duration of certainly not less than 1 microsecond, but in most cases 10–100 times more. This is, roughly speaking, the time for which the cathode spots have been observed to be apparently steady in the high-speed photographs. The noise measurements are therefore compatible with the assumption that what we see in the photographs are real diffuse spots, steady for perhaps 0·01–0·1 millisecond. They are scarcely compatible with the explanation in terms of much smaller spots, moving about at high speeds. One could expect such small spots to cross their own path, and those of others, fairly often before the mercury film has re-established itself, and this would lead to rapid breaks in the current, which ought to have revealed itself as high-frequency noise.

The provisional conclusion from these experiments therefore appears to be that though the Bertele discharge is not an even, uniform glow, and at any time covers only a part of the cathode area, its current density is not likely to be much more than a few hundred amp./cm.[2], and is probably steady over times of the order of at least 10 μsec. The experiments therefore suggest that cold metal cathodes can emit electron currents of this order, with macroscopic electric fields far too small to account for field emission, without high-pressure vapour jets, and with an ion current which is probably not more than a few per cent of the electron current. We do not propose a physical explanation of this phenomenon. We only note that the observations would be compatible with elementary discharges caused each by a single ion, breaking down the barrier over an area of some 10 Å. square only, provided that an ion can linger at a distance of a few Å. before the cathode surface for several microseconds, in a sort of metastable state of high energy. There is, of course, at present no theoretical support for such a contention.

We are indebted to Dr. A. Robson, Harwell, for a critical discussion of the experiments.

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Diffusion of Small Molecules

THE diffusion coefficient of a particle in solution is given by the Einstein equation[1]:

$$D = \frac{kT}{f} \tag{1}$$

where f is the frictional coefficient of the diffusing substance.

For spherical and rigid particles, large compared with molecules of the solvent, Stokes found[2]:

$$f = 6\pi\eta r$$

By introducing this relationship into equation (1) one obtains the Einstein–Stokes equation:

$$D = \frac{kT}{6\pi\eta r} \tag{2}$$

where η is the viscosity of the solvent, k the Boltzmann constant and r the radius of the diffusing sphere.

For spherical, but very small particles, Basset[3] has found the expression:

$$f = 6\pi\eta r \frac{\beta r + 2\eta}{\beta r + 3\eta}$$

where β is an empirical factor called 'coefficient of sliding friction' that ranges from zero to ∞. This introduces into equation (2) a correction factor ranging from 3/2 to 1.

Basset's equation has not yet been verified experimentally, however. Sutherland[4] carried out some diffusion measurements on alcohols and other organic substances and found that, for small molecules, the product Dr is greater than that calculated with equation (2) and decreases toward a constant value as the dimensions of the diffusing molecules increase. He considered that his measurements support the correction introduced by Basset.

This comparison is arbitrary indeed, if we consider that the model used by Stokes and Basset for their calculations is very different from a diffusing molecule, especially if it is linear instead of spherical, like alcohols and several other organic substances.

For diffusion measurements on linear and flexible molecules, we have found that, in some cases, equation (2) is verified if we substitute for r the gyration radius of the diffusing molecule to which is added the thickness of the atomic groups —CH_2— or —OH— constituting the molecule (that is, the radius corresponding to the action field of these groups). We have found that in these cases such thickness is 0·95 Å. We shall call s the sum of the gyration radius and the atomic thickness; for calculations refer to our earlier papers[5,6]. We have stated that: (*a*) when solute molecules are large compared to those of the solvent, as in the case of water solutions of organic molecules or for benzene solution of polymers, equation (2) is valid; (*b*) when solute molecules are of the same size as those of the solvent, as for benzene solutions of alcohols, esters and hydrocarbons, the radius calculated by equation (2) is smaller than the theoretical gyration radius s. The difference becomes greater when the solute and the solvent sizes are similar, and approaches zero with increasing dimensions of the solute.

Even if there is not as yet any theory relating the diffusion coefficient to the gyration radius, it is, however, interesting to note that in two cases at least, when the ratio of the dimensions of solute and solvent is great enough, equation (2) can be applied. We can, therefore, argue that a theory founded on a more realistic model should give an expression similar to equation (2).

We have used this expression to calculate the gyration radius of linear polymers in benzene, obtaining values agreeing with results using other methods[7].

In this communication we discuss some measurements carried out in the case where equation (2) is not valid, that is, when solute molecules are smaller, equal to or a little larger than those of the solvent. We have found that in this range the values of r (calculated by equation (2) from the measured values of D) are smaller than the values of s, calculated from the molecular structure.

We shall introduce the ratio s/r as a parameter to study that behaviour. Such a ratio in the case of spherical particles of Stokes's model corresponds to the correction factor calculated by Basset.

Measurements of diffusion coefficient were carried out in two solvents at 20 ± 0·01° C. by means of a method described before[6]. The precision of measurement was 0·5 per cent and the reproducibility about 1–2 per cent.

The products used were from the best firms, purified when necessary and identified by chromatographic analysis, refractive index and melting point.

The solvents were benzene and n-heptane; for the former we have assumed the value $s_{0,1} = 2{\cdot}35$ Å. $(1{\cdot}4 + 0{\cdot}95)$ and for the latter $s_{0,2} = 3{\cdot}0$ Å. $(2{\cdot}05 + 0{\cdot}95)$.

For the measurements in benzene see ref. 5; we list the measurements for n-heptane in Table 1.

Table 1

Substances	$D \times 10^7$ (cm.²/sec.)	r (Å.)	s (Å.)	s/r
n-Decane	206	2·50	3·50	1·40
n-Tetradecane	165	3·12	4·06	1·30
n-Octadecane	146	3·53	4·53	1·28
n-Octacosane	108	4·78	5·51	1·15
n-Dotriacontane	100	5·16	5·83	1·13
Methylcaprate	204	2·52	3·50	1·39
Methylcaproate	182	2·83	3·80	1·34
Ethyl ether	285	1·81	2·44	1·34
Dimethylketone	312	1·65	2·05	1·24

Fig. 1 shows values of s/r, measured in the two solvents, as a function of s.

We note that both behaviours are similar. In either case the values of the ratio rise to a maximum and then approach 1 asymptotically for high values of s. This confirms that equation (2) is useful for calculating the gyration radius of high molecular weight molecules like polymers with good precision, perhaps the best among the methods known nowadays. It is very interesting to point out the existence of a maximum in both cases; it was perhaps unexpected that, in a range before the maximum, smaller molecules diffuse more slowly than large ones. It is remarkable that in both cases the abscissa of the maximum is approximately the value of the gyration radius of the solvent (about 2·35 for benzene and 3 for n-heptane). A change in the rate of diffusion occurs when the solute molecules reach the dimensions of the solvent. In other words, molecules smaller than solvent molecules diffuse slower the smaller they are, while solute molecules larger than those of the solvent behave according to the Einstein–Stokes equation.

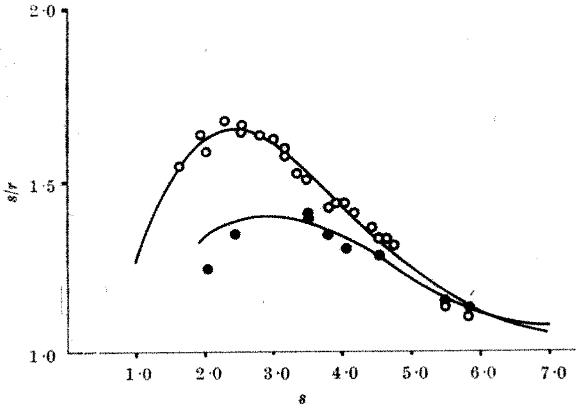

Fig. 1. ○, Measurements in benzene; ●, measurements in n-heptane

These facts are certainly related to the structure of the liquid state and to the diffusion mechanism. Therefore we think that our measurements and results can provide an interesting basis for theoretical work.

It would be useful to extend the measurements to a greater number of solvents and solutes, but, for the moment, we have limited our studies to the above cases owing to the difficulty of finding proper solvents and solutes.

We have also tried to find an empirical relation which predicts experimental results. In previous work[5] we have written an equation, very easy to apply, which accounts only for the latter part of the curve for benzene solutions and was therefore insufficient from a general point of view.

The distribution of the points and the presence of a maximum make us foresee an exponential behaviour. By trial we have found the relations:

$$s/r = 1 + 0{\cdot}90\, s^3 \exp(-1{\cdot}23 s) \quad \text{for benzene}$$

$$s/r = 1 + 0{\cdot}33\, s^3 \exp(-1{\cdot}04 s) \quad \text{for } n\text{-heptane}$$

which are shown as full lines in Fig. 1. As we see, they agree sufficiently with experimental values.

We have tried to formulate a single expression which includes these relationships by using as a parameter the dimension s_0 of the solvent molecules.

The two relationships may be written in the general way:

$$s/r = 1 + A_1 s^3 \exp(-B_1 s)$$
$$s/r = 1 + A_2 s^3 \exp(-B_2 s)$$

We have to examine whether there is a relationship between the two constants A_1 and B_1 of benzene, A_2 and B_2 of n-heptane and the dimension s_0 of the solvent molecules.

We have found that we can write:

$$A = A_1 s_{0,1}^4 = 27{\cdot}4 \qquad B = B_1 s_{0,1} = 2{\cdot}89$$

$$A = A_2 s_{0,2}^4 = 26{\cdot}7 \qquad B = B_2 s_{0,2} = 3{\cdot}12$$

The two values of A and the two of B are so close as to be considered two constants independent of s_0. This agreement is satisfactory if we take into account that the values found depend on precision of measurement of the diffusion coefficient and on the theoretical calculation of the gyration radius.

We can therefore suggest a parametric relation:

$$s/r = 1 + (A/s_0^4) s^3 \exp(-B/s_0) s$$

where A has a value of about 27 and B a value of about 3.

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Anomalous Conductivity and Lorenz Parameter in Dilute Silver–Manganese Alloys at Low Temperatures

Low-temperature electrical resistivity measurements by Gerritsen and Linde[1] on silver alloys containing small amounts of manganese in solid solution showed that, instead of attaining a temperature-independent 'residual' resistivity, they presented a minimum somewhat below liquid-hydrogen temperatures, followed by a maximum at still lower temperatures.

Measurements of the thermal conductivity (λ) of three dilute silver–manganese rods containing respectively 0·55, 0·32 and 0·14 atoms per cent of manganese revealed[2,3] an anomalous behaviour at liquid-helium temperatures in a zero magnetic field. This anomaly consisted in the $\lambda : T$ curve exhibiting a 'knee' at about 2·7° K., so that the values of λ below the knee were higher than would result if the curve above the knee were simply extended to the lower temperatures. The $\lambda/T : T$ curve exhibited this anomaly in the form of a 'dip' followed by a 'hump' at the lowest temperatures[2,4].

Since the shape of the $\lambda : T$ curve was not significantly altered by magnetic fields as high as 25 kilogauss, it was suggested that the zero-field thermal conductivity anomaly might be due to an extra contribution to the lattice thermal conductivity, owing probably to a partial uncoupling of the longitudinal and transverse phonons[2,5]. However, it was not understood why the $\lambda/T : T$ curves 'dipped' below the normal rectilinear behaviour, at temperatures between about 2·5 and 5° K. (Fig. 2, in ref. 4).

It has now been possible to analyse the thermal and electrical conductivity data on these alloys, in magnetic fields and in zero field, by the method of Gruneisen–de Haas[6]. The plots of $\lambda_{[H]} : \sigma_{[H]}T$ at any temperature (where $\lambda_{[H]}$ and $\sigma_{[H]}$ are the values of the thermal and electrical conductivities for different values of the transverse magnetic field H, including $H = 0$) can be considered as straight lines. This means that the electronic Lorenz parameter L_e seems to be independent of the magnetic field-strength. However, it exhibits also an interesting variation with temperature. It is found that, in these alloys, at liquid-helium temperatures: (1) L_e is less than the 'normal' Sommerfeld value ($2 \cdot 45 \times 10^{-8}$ watt-ohms-deg.$^{-2}$), but approaches that value at about 1° K. or lower; (2) L_e has a minimum value at a temperature $T_{\text{min.}}$; (3) $T_{\text{min.}}$ varies with the manganese content c, shifting to a higher temperature with increasing c, and (4) the depth of the minimum is reduced with increase of c.

The fact that L_e is less than the Sommerfeld value at liquid-helium temperatures accounts for the 'dip' in the $\lambda/T : T$ curves for these alloys, between about 2·5 and 5° K.

Full details will be published elsewhere.

Grateful acknowledgment is made of the benefit of discussions with the director, Sir K. S. Krishnan.

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Aggregation and Dispersal of Radiation Damage in Graphite

Following pioneering work by Grenall[1], Williamson[2] has used the technique of transmission electron microscopy to elucidate some aspects of the behaviour of dislocations in natural crystals of graphite, and Amelinckx and Delavignette[3] have demonstrated the formation of vacancy loops in quenched material. We have repeated some of these observations and applied the method to the study of neutron irradiation damage and its behaviour in crystals annealed up to 2,500° C. Our results appear to be consistent with those of Bollmann[4], who obtained photographs of defects in artificial graphite annealed up to 400° C.

Crystals of natural graphite obtained from Ticonderoga calcite were irradiated in the high-flux reactors *Dido* and *Pluto* to doses of about $4 \cdot 8 \times 10^{20}$ neutrons per sq. cm. (~500 equivalent megawatt-days per tonne (of fuel)) at various temperatures in the range 150–650° C. It was found advisable to give the crystals a purifying heat treatment at 1,500° C. before irradiation. Even then, however, the unirradiated material showed some flaws, including lenticular formations with diameters in the range 0·1–3μ similar to those first seen by Rang[5] in mica. Specimens were prepared by repeated cleavage using adhesive tape, and were examined in a Siemens Elmiskop microscope at 100-kV. anode voltage.

The general behaviour of the damage effects seen is illustrated in Figs. 1 and 2, which are on a scale of 120,000 : 1. The material of Fig. 1*a* was irradiated at 200° C. and the damage is present as very fine speckling, barely resolved but quite clear in the neighbourhood of an extinction contour. The light and dark contrast is of no particular significance, as the same feature changes from one to the other when the contour is moved by tilting the specimen. At higher temperatures, damage sites are larger, rounder and more sparse, as illustrated in Fig. 1*b*, which was obtained by irradiation at 650° C. Dislocations have not been found on material irradiated at the lower temperatures and are difficult to see on material irradiated at the higher temperatures. Dark-field images show no additional features.

Annealing of the low-temperature damage causes the defects to form clusters, the effect becoming marked at about 1,000° C. as shown in Fig. 1*c*. The clusters

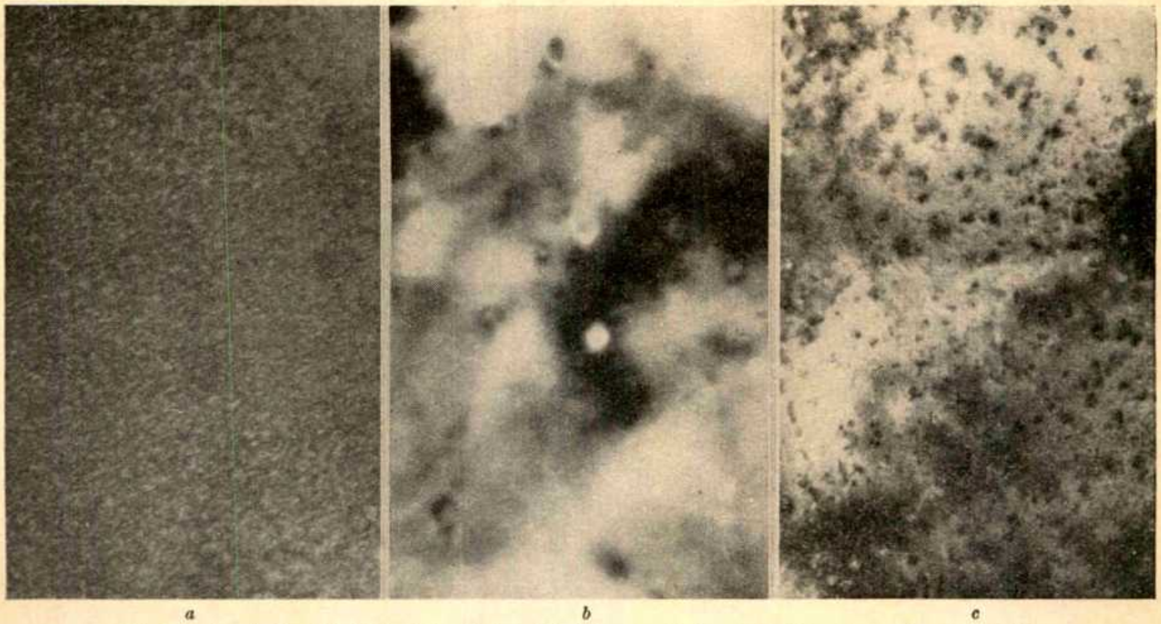

Fig. 1. *a*, Irradiation at 200° C.; *b*, irradiation at 650° C.; *c*, irradiation at 200° C. and annealing at 1,000° C.

appear to be very complex, and they are very irregular in shape, each one being apparently made up of a number of smaller units which are probably in different planes. It is possible that these clusters are formed by the migrations of smaller groups, but more likely that they are formed by the preferential growth of some sites as the less-stable ones break up. The process continues up to 1,500° C., as illustrated by the large clusters in Fig. 2*a*. Sometimes these large clusters align themselves in particular directions, as shown in Fig. 2*b*; but these directions have not been found to correspond to any particular crystal lattice vector as tested by the local electron diffraction pattern. They are therefore assumed to be associated with a local dislocation array. A few examples have also been found of alignment on parallel smooth curves. In other regions, the clusters spread out roughly along the hexagonal sides of a 'chicken-wire' pattern, as in Fig. 2*c*, suggesting that possibly ascertainable fields of force exist between complexes.

Damage incurred at 150° C. begins to disappear rapidly at annealing temperatures above 1,500° C. At 1,550° C., many of the larger clusters have been broken up, and at 1,700° C. there is little evidence of damage except a possibly enhanced dislocation density. Specimens annealed at higher temperatures are indistinguishable from unirradiated material, but there is some evidence that material irradiated at 200° C. requires slightly higher temperatures for complete annealing.

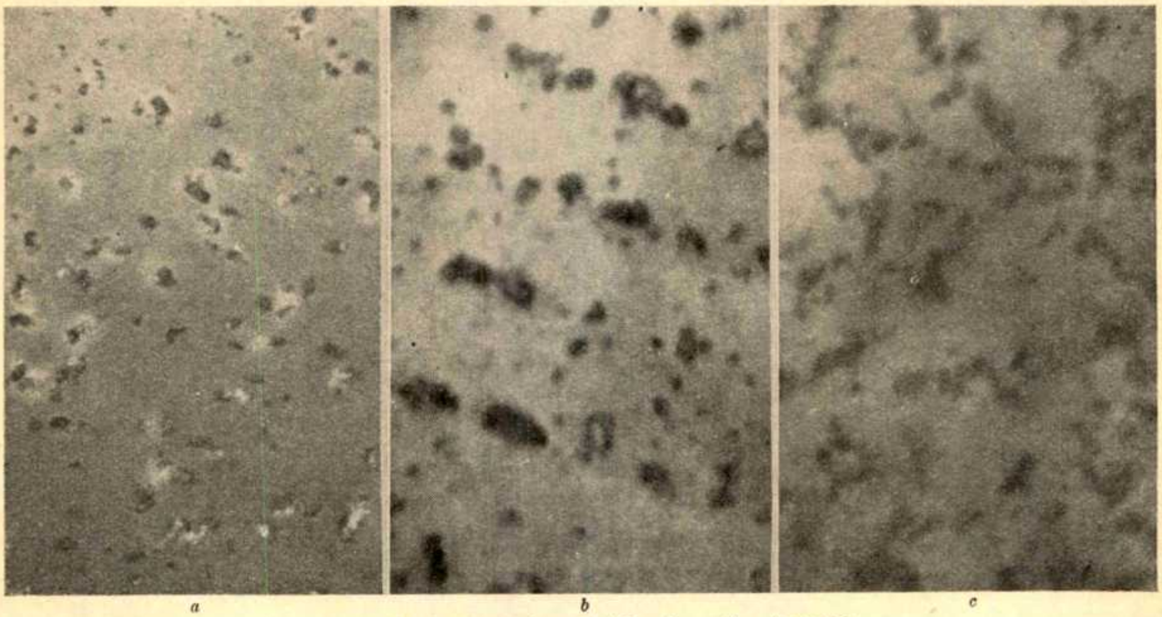

Fig. 2. *a*, *b* and *c*, Irradiation at 200° C. and annealing at 1,500° C.

It is not yet possible to say definitely whether the damage seen is predominantly due to interstitial atoms or vacancies, or if both kinds of defect are involved. However, interstitials are thought to be more mobile at least up to 1,000° C., and the general behaviour does not seem to correspond to that of vacancies as described by Amelinckx and Delavignette[3], so that it may be assumed provisionally that the effects constitute a direct observation of the behaviour of interstitial clusters.

General studies of neutron-irradiated graphite (see, for example, Simmons[6]) have already suggested the formation of highly stable complexes by the annealing of low-temperature damage, and more detailed studies will now be possible. The rapid disappearance of defects above 1,500° C. corresponds well with the final annealing of electrical effects observed in unpublished work by Eatherly.

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GEOPHYSICS

Auroral Observations at Halley Bay, Antarctica, during 1959

IN accordance with the recommendations of the Special Committee on Antarctic Research the programme of auroral observation conducted during the International Geophysical Year at Halley Bay, Antarctica, was continued into 1959. The Halley Bay Base, established by the Royal Society International Geophysical Year Expedition and now administered by the Falkland Islands Dependencies Survey, is situated in geomagnetic latitude 66° S., and both visual and all-sky camera records are now available for the four years 1956–59. A detailed analysis of the results of the first three years observations has appeared elsewhere[1], and it is interesting to compare these results with those obtained during 1959.

Of particular interest is the apparent change in the position of the southern auroral zone over the course of the four years which includes the period of sunspot maximum in 1957. Previously[2] the position of a 'quiet arc zone' has been defined in terms of the median value and interquartile range of the latitudinal distribution of quiet arcs. Such a distribution has now been obtained using the results of the 1959 observations; the positions of the quiet arc zone for the four consecutive years are given in Table 1.

Table 1

Year	1956	1957	1958	1959
Median latitude (°S. geomagnetic)	70·8	71·8	71·9	71·0
Interquartile range	±1·3	±1·0	±1·0	±1·1

Paton[3] has recently suggested that there may be a simple geometrical connexion between the position of the quiet arc zones in the two hemispheres and the 'horns' of the Van Allen belts of geomagnetically trapped radiation. The changes in the character of these belts during the period July 1958–August 1959[4] are certainly in keeping with this proposal.

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METALLURGY

Theory of the Deformation and Fracture of Body-centred Cubic Transition Metals

EXPERIMENTS on a number of body-centred cubic transition metals[1] have shown that, above the ductile–brittle transition temperature, the yield stress, σ_y, varies with the average grain diameter, $2d$, according to the relation:

$$\sigma_y = \sigma_i + k_y d^{-1/2} \qquad (1)$$

Below the transition temperature, the brittle fracture stress, σ_f, is related to the grain size by the similar relation:

$$\sigma_f = \sigma_i + k_f d^{-1/2} \qquad (2)$$

Cottrell[2] has developed a theory of the ductile–brittle transition in terms of the parameters σ_i and k_y. According to the model adopted by Cottrell, σ_i is a lattice friction stress which contains a temperature-dependent part tentatively attributed to the Peierls–Nabarro stress of the lattice. The grain size parameter, k_y, is related to the stress, σ_d, required to unpin a dislocation from its impurity atmosphere and is given by the equation:

$$k_y = \sigma_d l^{*1/2} \qquad (3)$$

where l^* is the average distance from a grain boundary to the nearest dislocation source. In developing his theory Cottrell found that for a specimen to be brittle the criterion:

$$(\sigma_i d^{1/2} + k_y)k_y \geqslant \beta\mu\gamma \qquad (4)$$

must be satisfied. Here β is a constant which is nearly unity, for uniaxial tension, μ is the rigidity modulus, and γ the effective surface energy for fracture. The relations between the brittle fracture stress, the stress for ductile cleavage fracture, $\sigma_{f'}$, and the grain size which he obtained were:

$$\sigma_f(\sigma_f - \sigma_i)d = \beta\mu\gamma \qquad (5)$$

and

$$\sigma_{f'} = \frac{\beta\mu\gamma}{k_y} d^{-1/2} \qquad (6)$$

These equations appeared, at the time that the theory was formulated, to agree with most of the available experimental results, although no adequate results existed to test equation (6). Equation (5) is not significantly different from equation (2) over the ranges of grain size and temperature so far studied.

Recent experimental work in this laboratory and elsewhere has revealed several inadequacies in the theory of which three will be considered. First, a set of measurements on molybdenum at − 78° C. (ref. 3) has shown that the dependence of ductile cleavage fracture stress on grain size is not correctly described by equation (6). Secondly, it has been found that the value of k_y for niobium is very small or zero at all temperatures[1]. This suggests, from equation (3), that the dislocation locking in niobium is very weak. On the other hand, sharp yielding is observed in niobium up to about 400° C. (ref. 4), and this suggests that the locking is at least as strong as in iron. Thirdly, since k_y is proportional to the unpinning stress, it should be strain-rate sensitive, whereas a recent set of measurements on iron and steel[5] have shown that it is not.

In order to explain these discrepancies between the experimental results and Cottrell's theory, I have found it necessary to develop a modified form of the theory. This has involved a re-interpretation of the parameters σ_i and k_y. Since a full account of this theory will be published elsewhere, only an outline of the main conclusions reached will be given here.

It has been established that the parameter σ_i is the sum of two terms, $\sigma_i(\mathrm{I})$ and $\sigma_i(L)$, that is :

$$\sigma_i = \sigma_i(\mathrm{I}) + \sigma_i(L) \qquad (7)$$

where $\sigma_i(\mathrm{I})$ is a lattice friction stress due to impurities and $\sigma_i(L)$ is the stress required to propagate a Luders band through a single crystal having no lattice friction stress. The term $\sigma_i(L)$ is a function of the unpinning stress, σ_d, and of the work-hardening properties of the metal, and it is therefore strongly temperature dependent. The Peierls–Nabarro stress does not contribute significantly to σ_i. The parameter k_y is a measure of the work-hardening which occurs near grain boundaries during the propagation of a Luders band in a polycrystal. It is determined mainly by the work-hardening properties of the metal, but is also influenced by the unpinning stress, σ_d.

The treatment of brittle fracture and the ductile–brittle transition given in the theory is based on the observation that, at and above the transition temperature, a growing microcrack is unable to penetrate the region of forced slip near the boundary of the grain in which it is nucleated. The criterion for brittle–fracture is then :

$$\sigma_i + k_y d^{-1/2} + \Delta\sigma \geqslant \sqrt{\frac{2\gamma_e E}{\pi(d - d_0)}} \qquad (8)$$

Here $\Delta\sigma$ is the magnitude of the yield drop, γ_e is the effective surface energy for fracture, E is Young's modulus, and d_0 is the distance from the tip of an arrested crack to the grain boundary. The values of γ_e calculated from equation (8) vary from metal to metal and for a given metal depend on grain size. They lie in the range $8{\cdot}5 \times 10^3$ to $6{\cdot}5 \times 10^5$ ergs cm.$^{-2}$.

The interpretation of these values of γ_e leads to the conclusion that for a body-centred cubic transition metal to be relatively brittle it must have both strongly locked dislocations and a high work-hardening rate. The transition temperature comes out to be roughly independent of grain size for brittle metals, such as tungsten and molybdenum, but strongly dependent on grain size for relatively ductile metals, such as niobium and steel.

The theory has also been used to reinterpret existing experimental results on the hardening and embrittlement of mild steel[6] and molybdenum[7] by neutron irradiation. It is found that, as a result of irradiating mild steel near room temperature, both the friction stress, $\sigma_i(\mathrm{I})$, and the unpinning stress, σ_d, increase. In the case of molybdenum, only σ_d increases significantly. The deviation from equation (1) shown by irradiated molybdenum is shown to be the consequence of a combination of a particularly high unpinning stress and a high work-hardening rate. The theory predicts that the increase in transition temperature caused by irradiating mild steel should be greatest in specimens having a fine grain size, and that for large neutron doses the transition temperature should become independent of grain size.

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CHEMISTRY

Electronic Properties of Phenazine

MANY organic compounds such as naphthalene, anthracene, etc., have extremely small, but detectable, electrical conductivities, which increase exponentially with an increase in temperature according to the equation $\sigma = \sigma_0 \exp(-E/2kT)$, where σ is the specific resistivity, σ_0 is a constant, E is an activation energy generally referred to as the energy gap and T is the absolute temperature. Since it is generally held that the conductivity is electronic rather than ionic, these substances have been termed organic semiconductors[1]. Lyons[2] has pointed out that a correlation exists between the magnitude of the energy gap and a number of molecular properties, such as the molecular ionization energy, the molecular electron affinity, and the excitation energy of the ground-triplet state transition. If it is assumed that the semiconductivity of organic compounds involves the formation of biradicals and hence participation of the triplet state in the conduction process, it becomes evident that the energy gap should be at least equal to the ground-triplet state excitation energy. Of particular interest is the fact that for a number of compounds the energy gap is roughly equal to (generally slightly larger than) the aforementioned excitation energy. Thus, the energy gaps[3] for anthracene, naphthacene, chrysene, pyrene, and anthanthrene are, respectively, 1·95, 1·64, 2·04, 2·06, and 1·58 eV., which are in fair agreement with the ground-triplet excitation energies of 1·82 [4], 1·26 [5], 2·45 [4], 2·08 [3], and 1·32 [3] eV.

We have now found that this relationship apparently also holds true for the heterocyclic compound phenazine. From the dependence on temperature of the resistivity (Fig. 1*a*) of phenazine in the range of 98–143° C., we determined the energy gap as 2·1 eV. This compares favourably with the ground-triplet excitation energy[6] of 1·9 eV. The resistivity was determined in an atmosphere of dry nitrogen on a disk of phenazine which was prepared by compaction

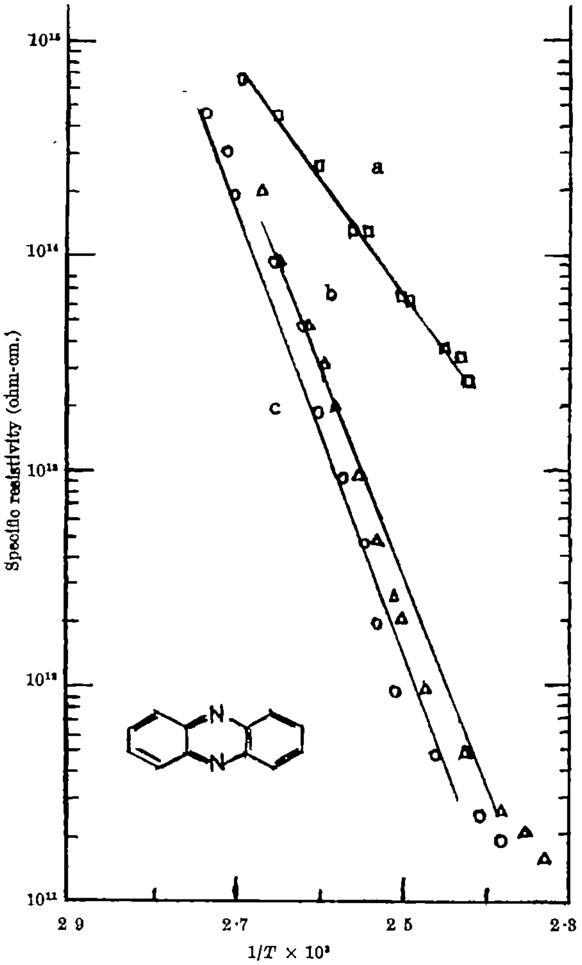

Fig. 1. Specific resistivity of phenazine: *a*, □, zone-refined, 94 and 940 V./cm.; *b*, △, two sublimations, 68 V./cm.; *c*, ○, three sublimations, 67 V./cm.

under high pressure and which was sandwiched between mercury electrodes during the measurements.

There is no *a priori* reason for suspecting that the energy gap obtained from measurements on a compacted polycrystalline material is different from that of a single crystal. Although the possibility of the presence of intergranular resistances in compacted specimens cannot be ruled out, these resistances should not be more serious than those possibly present in evaporated films. Northrop[3] has shown that whereas evaporated films of hydrocarbons have higher specific resistivities than single crystals, both have the same energy gap within experimental error. It is therefore apparent that reliable and theoretically significant energy gaps (albeit not specific resistivities) can be derived from measurements on suitably prepared void-free polycrystalline materials.

The phenazine was purified by chromatography on alumina and subsequent zone-refining, but estimation of the degree of purity was not attempted.

While the process of zone-refining in itself does not constitute evidence of 'hyperpurity', results obtained on conventionally purified phenazine lend support to the assumption that a specimen of high purity was on hand. Figs. 1*b* and 1*c* show, respectively, the resistivity of phenazine which, after chromatography, had been sublimed two and three times in a high vacuum. Both specimens display lower resistivities, particularly at the higher temperatures, and have energy gaps of 3·8–4·0 eV. As it is unlikely that the zone-refined specimen contained a larger number of inert impurities or physical voids than the sublimed specimens, it may be concluded that the higher specific resistivity and lower energy gap obtained in the former are consequences of its greater state of purity.

A detailed account of this work will be published elsewhere. It was supported by the Air Research and Development Command, United States Air Force, under Contract No. *AF* 33(616)–6908 monitored by the Materials Laboratory, Wright Air Development Division, Wright-Patterson Air Force Base, Ohio.

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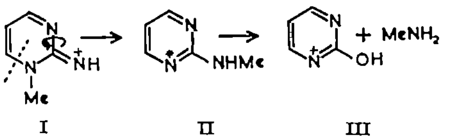
Apparent Migration of N-Methyl Groups in the Pyrimidine Series

The long-standing problem of how methyl groups migrate from nuclear to extra-nuclear nitrogen atoms in heterocyclic compounds has now been decided experimentally in the pyrimidine series.

Methyl iodide reacts[1] with 2-aminopyrimidine to give the hydroiodide of 1,2-dihydro-2-imino-1-methylpyrimidine (I). The free base may be liberated with cold sodium hydroxide, but if the alkaline solution is warmed, rearrangement to 2-methylaminopyrimidine (II) is complete in 10 min.[1,2]. Similar rearrangements are known to occur under both alkaline and acidic conditions in the pyrimidine[3–6], pyridine[7,8], pteridine[9], purine[10], and other[11,12] series.

Two facts of possible significance in connexion with the mechanism of such isomerizations are: (*a*) Rearrangement occurs only when the methyl group and the amino- or imino-group are attached to neighbouring nuclear atoms, and never when they are placed otherwise. (*b*) A nuclear-N-methylated methylaminopyrimidine failed[5] to yield a dimethylaminopyrimidine under conditions where this might reasonably be expected to happen. These facts suggest a hypothesis of hydrolytic ring fission followed by rotation and recyclization. Thus, for example, the rather polarized 1,6-bond in compound (I) would break, allowing rotation about the 2,3-bond and recyclization to the almost aromatically stable compound (II). If this hypothesis is correct, the extra-nuclear nitrogen atom in compound (I) would become a nuclear atom of compound (II).



I II III

To test the hypothesis by this means, 2-chloropyrimidine was heated with ammonia (enriched by nitrogen-15) in ethanol for 2 hr. at 120° C. to give 2-aminopyrimidine labelled in the amino group. This was treated with methyl iodide[1], and the resulting compound (I) (hydroiodide, of melting point 251° C. undepressed by admixture with unlabelled material) was rearranged[1] in hot alkali to 2-methylaminopyrimidine (II). Hydrolysis of the sublimed material (melting point 58° C.; published 59–60° C.) with *N* hydrochloric acid at 160° C. for 6 hr. gave 2-hydroxypyrimidine (III) (melting point 178° C.; published[13] 178–80° C.) and after making the residual solution alkaline, methylamine was isolated using steam-distillation and collected as its picrate (melting point 207° C., undepressed by authentic material). Mass-spectrometric examination of nitrogen samples prepared by burning compound (III) and the methylamine picrate showed a strong nitrogen-15 enrichment in nitrogen from the 2-hydroxypyrimidine, but none in nitrogen from the picrate. The rotation hypothesis for the mechanism of rearrangement was thus valid.

Mr. J. Harper and Mr. B. T. England assisted in the experimental work, and the mass-spectrometric examination was kindly made by Cmdr. J. Mason in the University of New South Wales.

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The Sodium Silicate – Ferrous Hydroxide System

We have reported earlier[1] the occurrence of a point of inflexion at *p*H 10·75 on the hydrolysis curve of a 0·01 *M* sodium silicate solution which was attributed to the formation of the trihydroxy – *ortho*silicate ion, but we were unable to detect with certainty a point on this curve corresponding to the final decomposition of this ion to *ortho*silicic acid, $Si(OH)_4$. An investigation of the sodium silicate – ferrous hydroxide system has shed new light on this problem. It is known that controlled oxidation of ferrous hydroxide between *p*H 5·2 and 11·5 yields magnetite[2,3], and it has also been established[4] that silica prevents the formation of magnetite from solution. One could therefore expect a systematic investigation of the sodium silicate – ferrous hydroxide system over a wide *p*H range to assist in clarifying the behaviour of silicic acid.

The experimental procedure involved addition of sodium silicate solutions to 0·005 *M* suspension of precipitated ferrous hydroxide, yielding a final ratio of sodium silicate to ferrous hydroxide of 0·4 : 1 (excess ferrous hydroxide) and 1·5 : 1 (excess silicate) respectively, and subsequent oxidation of two-thirds of the iron present with 0·3 per cent hydrogen peroxide; *p*H of the mixture was varied between 5·1 and 13·8 and the entire operation carried out under a nitrogen atmosphere at 25 ± 0·02° C. Samples were tested for magnetic behaviour and submitted to X-ray analysis using the powder diffraction method.

We found that, below about *p*H 10·7, magnetite is not formed in any appreciable quantities, whereas above this point it is the major product together with some very finely divided α-ferric oxide. On the other hand, an analogous procedure in the absence of silicate ions led us to establish *p*H 5·25 as the lower limit for the formation of magnetite.

It would therefore appear that *p*H 10·7 marks the end of hydrolysis of the dihydroxy – *ortho*silicate ion in 0·002–0·007 *M* sodium silicate solutions:

$$(SiO_2(OH)_2)^{2-} + 2H_2O = Si(OH)_4 + 2OH^-$$

Such freshly formed silicic acid in aqueous solution, being in an active condition[5], will be readily adsorbed by ferrous hydroxide particles and thus prevent the formation of magnetite below *p*H 10·7. This finding can be related to a recent observation[6] that at *p*H 8·5 the formation of magnetite from solution is a solid phase reaction.

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Reaction of Phosphorylating Agents with 2-Methyl-Δ^2-Oxazoline

Degradation of esteratic enzymes which have been inhibited with di*iso*propyl phosphorofluoridate (DFP) yields serine phosphate[1], although serine itself is completely unreactive to DFP[2]. Kinetic and other evidence[3] suggests participation of the imidazole ring of a histidine moiety in the phosphorylation reaction, although it is not known if the ring is the initial site of phosphorylation or whether it catalyses phosphorylation of the serine. Rydon[4] has postulated that a Δ^2-oxazoline is present in the enzyme which on phosphorylation yields an O-phosphoryl serine residue. This hypothesis was based on the results of Porter *et al.*[5], who showed that 2-aminoethyl phosphate could be isolated from the reaction of 2-methyl-Δ^2-oxazoline (I) and DFP in aqueous solution. Since acylating agents[6] react with I to give stable N-acyl derivatives, and since 2-aminoethyl phosphate was isolated by Porter *et al.* following acid hydrolysis of the product (conditions under which N → O migration of phosphoryl groups is known to occur[7]) the reaction of I with phosphorylating agents was further examined.

An aqueous solution of I buffered at *p*H 8·2 with sodium bicarbonate reacted with an equimolar amount of di*iso*propyl phosphorochloridate (DClP) to give, after stirring for 24 hr. at room temperature, di*iso*propyl 2-acetoxyethylphosphoramidate in 51 per cent yield. Under these conditions no phosphorylated products were obtained with DFP. When the experiment was repeated with a three-fold excess of DFP under the conditions employed by Porter *et al.* (*p*H 7·8, 37°, 4 days with *iso*propanol added to homogenize the system[8]) two products were isolated from a chloroform extract by chromatography on Woelm IV neutral alumina followed by quantitative electrophoretic separation. They were identified as 2-aminoethyl di*iso*propyl phosphate (0·07 per cent) and 2-acetamidoethyl di*iso*propyl phosphate (0·08 per cent) by means of their infra-red spectra and their behaviour on paper chromatography and electrophoresis. Using these latter conditions the reaction of DClP with I gave di*iso*propyl 2-acetoxyethylphosphoramidate (20 per cent) and di*iso*propyl 2-hydroxyethylphosphoramidate (0·4 per cent). It was found that I was almost completely converted to N-acetyl ethanolamine (II) after 3½ days in water/*iso*propanol buffered at *p*H 7·8 with sodium bicarbonate. It therefore seemed feasible that the O-phosphoryl products obtained from an aqueous solution of I and DFP could have resulted from the side reaction of II with DFP. Experiments did in fact show that, under identical conditions, II reacted with DFP giving 2-acetamidoethyl di*iso*propyl phosphate (0·35 per cent).

These results indicate that the O-phosphoryl derivatives obtained from aqueous solutions of I and DFP are not formed by N → O migration during the isolation procedure. However, it is difficult to explain why DFP should react with I to give an O-phosphoryl derivative although DClP yields a stable N-phosphoryl derivative as would be expected from the analogous reaction of acylating agents. The reaction of II with DFP giving O-phosphoryl compounds may provide an explanation of the anomaly. These facts should be borne in mind while considering the hypothesis of serine being present in the active site of esteratic enzymes as an oxazoline ring.

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Enhancement of Atomic Absorption in the Flame by Organic Solvents

ATOMIC absorption methods for the determination of many cations have attracted much attention[1,2] lately. The techniques so far described usually involve the use of a flame for the production of a population of atoms, but the method is of wider application than emission flame photometry and seems to be free of some, though by no means all, of the inter-element effects that bedevil emission methods. The sensitivities obtained are high, especially for certain elements, for example, magnesium, zinc, but for others, particularly in specialized applications, for example, calcium in biological materials, a higher performance is desirable, and it is therefore of interest to look for ways of enhancing sensitivity. We have found that an improvement of 100–1,000 per cent (depending on the element under assay) can frequently be achieved if the test solutions are made to contain a high (⋗ 50 per cent v/v) concentration of an organic solvent such as propanol or acetone.

Table 1 shows the effect of a variety of alcohols at several concentrations on the atomic absorption resulting from spraying a standard magnesium chloride solution containing 2·4 p.p.m. magnesium. It will be observed that the readings increase with increasing amounts of alcohol, but that the initial small additions have a proportionally greater effect. This initial effect is more marked as the chain-length increases.

Table 1

Organic solvent (per cent)	Organic solvent				
	MeOH	EtOH	PrOH	BuOH	AmOH
0	0·187	0·181	0·185	0 180	0·180
15			0·332	(8 per cent) 0·348	(2 per cent) 0·270
33	0·326	0 389	0·447		
50	0·390	0·448	0·447		
66	0·450	0·502	0·447		

Flame coal gas, 15 lb./sq. in. in air from cylinder. Slit width, 0 2 mm. Line, 2852 Å. 2·4 p.p.m. magnesium ions. Each series sprayed three times. Range of reading ± 3 per cent.

Similar results are found with other elements, and Table 2 shows the enhancement we have found, for the elements listed, in 50 per cent *iso*propanol solution. We have found this a convenient solvent to use because the enhancement reaches a plateau at this concentration of propanol or *iso*propanol, hence there is no point in exceeding it, but the flame stability was rather better with the branched-chain alcohol.

Table 2

Element sought	Line	Atomic absorption in 50 per cent *iso*propanol is enhanced by a factor of (approximately)
Ni	3414	2
Cs	8521	1·3
Ag	3281	2
Ca	4226	3
Fe	2483	8–10
Zn	2138	2

The reason for the increased sensitivity is not clear. It is not due entirely to surface tension and viscosity changes, for experiment shows that neither the spray-rate nor the spray efficiency (that is, the fraction of the spray going forward into the flame) changes by anything like the amount required to account for the enhancement, while the addition of 'Aerosol *T*', a proprietary surface-active agent, had no effect. Moreover, the effect is quantitatively different from one element to another.

It is well known that organic solvents similarly enhance emission in the flame, and this has been attributed to increases in flame temperature, which is, of course, much more important in emission than in absorption. There is also the possibility that the production of free atoms is favoured by the change in flame chemistry brought about by the introduction of the organic solvent. This would be effective both in absorption and emission. We have therefore carried out the following experiment. The standard apparatus used for this work (Hilger *H*.700 spectrophotometer with *H*.909 atomic absorption attachment) was modified so that the burner could be fed with the mixed aerosol from two atomizers. Then: (1) an aqueous solution was sprayed; (2) a solution in 50 per cent propanol was sprayed; (3) an aqueous solution was sprayed from one atomizer and propanol from the other. Conditions were adjusted so that the amounts of test element, water, and alcohol actually entering the flame were the same in (2) and (3). The expected enhancement was found in (2) but not in (3). Since the amounts of alcohol and water entering the flame were similar in (2) and (3) it is reasonable to suppose that chemical and temperature conditions were similar. Clearly the explanation of the enhancement must be sought elsewhere. It seems possible that it is to be found in some change in the transient conditions that exist as the solvent in the droplets evaporates in the flame; but further elucidation is required. In the meantime, there is no doubt that a useful increase in sensitivity can be achieved if it is possible to work with organic solvents similar to those described. Other classes of compound show similar effects, for example, hydrocarbons, but are not recommended as they tend to cause instability of the flame.

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Isolation of L-Galactose-6-sulphate from a Seaweed Polysaccharide

Sulphated polysaccharides occur widely as major components of many seaweeds[1]. Until now, however, locating the sulphate hemi-ester groups in the polysaccharide structure has rested upon evidence from methylation data[2,3], supported in some cases by infra-red spectroscopy[4,5]. Here we report the application of the method of partial acidic hydrolysis to determine the position of sulphate residues in porphyran, the mucilage of the red seaweed *Porphyra umbilicalis*.

Our earlier work[6,7] has shown that porphyran contains residues of D-galactose, L-galactose, 6-O-methyl-L-galactose, 3,6-anhydro-L-galactose and ester sulphate respectively in the molar ratios 100 : 110 : 118 : 73 : 140. A similar polysaccharide has been isolated from *Porphyra capensis*[8]. The fact that the 3,6-anhydrogalactosidic linkages in these polysaccharides are very sensitive to hydrolysis in acid solutions[9,10] has prompted us to seek conditions under which glycosidic hydrolysis would proceed without the occurrence of any other changes in the polysaccharide structure. Degradation of the polysaccharide with 0·58 *N* sulphuric acid at 50 ± 1° C. for 10 hr. appeared to fulfil this condition. After this treatment, and neutralization with barium carbonate, the product was separated into fractions containing relatively high molecular weight and relatively low molecular weight substances. The fraction containing relatively high molecular weight products was isolated by precipitation from 90 per cent ethanol and subjected to further acid hydrolysis. Comparison of the rates of glycosidic and ester sulphate hydrolysis (Fig. 1) showed that after 5 hr., monosaccharide sulphates would be present in the hydrolysate, with very little contaminating oligosaccharides. It was calculated that about 27 per cent of the monosaccharide sulphate combined in the original polysaccharide could be recovered in this way. The sugar sulphate fraction was accordingly isolated from the hydrolysate by a combination of chromatography with thick paper[11], chromatography on a type of charcoal sufficiently active to retain sugar sulphates[12] and electrophoresis using a thick filter paper support and a volatile buffer[13]. The product, which was obtained as a syrup, was assigned the structure of L-galactose-6-sulphate on the following evidence: Equimolar quantities of galactose and sulphate were liberated on acid hydrolysis. The substance was shown to be a monosaccharide derivative by the method of Peat, Whelan and Roberts[14], and it moved as a single spot parallel to authentic D-galactose-6-sulphate on paper chromatography in a variety of solvent systems[11,15] and on paper electrophoresis. When treated with unbuffered sodium metaperiodate solution, 3·5 moles of oxidant[16] were consumed fairly rapidly, with no liberation of formaldehyde. This is identical with the behaviour of authentic D-galactose-6-sulphate[17]. Finally, the specific optical rotation $[(\alpha)_D = -47°$, sodium salt in water $c = 0{\cdot}15]$ was equal in magnitude, but opposite in sign, to the value for an authentic specimen of D-galactose-6-sulphate[11].

The fact that this 6-sulphate has been isolated as a hydrolysis product of porphyran cannot be taken as proof of its presence as a component of the original polysaccharide, unless it can be shown that sulphate ester migration has not occurred during hydrolysis. Although acyl migration of this type is well estab-

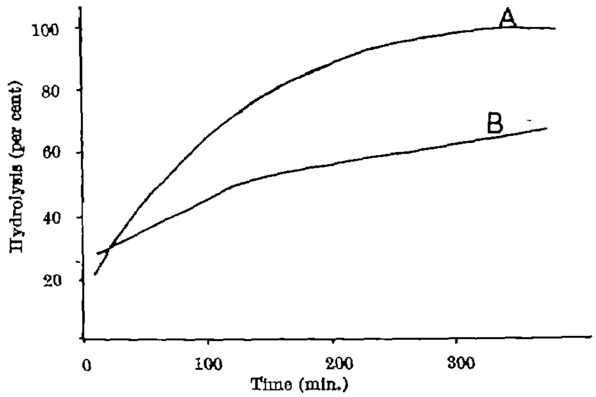

Fig. 1. Hydrolysis of oligosaccharide mixture with 0·24 *N* hydrochloric acid at 100° C. Increase in reducing power was estimated by the method of Somogyi (ref. 18) and sulphate liberation by the method of Jones and Letham (ref. 19)
A, glycosidic hydrolysis; *B*, sulphate ester hydrolysis

lished, for example, in the case of sugar acetates and sugar phosphates, it is considered unlikely here, in view of recent experiments in this laboratory with synthetic sugar sulphates. These experiments[20] have shown that when certain typical sugar sulphates (galactose-6-sulphate, galactose-4-sulphate, glucose-6-sulphate, and glucose-3-sulphate) are heated in dilute acid solution, the only reaction which can be detected by paper chromatography is the liberation of the parent sugar.

The analytical figures quoted above show that the number of sulphate ester groups present in the polysaccharide exceeds the number of L-galactose residues, and hence not all the sulphate in porphyran can occur as L-galactose-6-sulphate. Since only L-galactose-6-sulphate was isolated, it would seem that the other ester sulphates present are more acid labile. The inflexion in the curve representing the rate of sulphate release (Fig. 1) supports this view, and also suggests that more than half the total ester sulphate is liberated in the second phase of the hydrolysis at a slow rate, that is, is presumably released from the more acid-stable L-galactose 6-sulphate residues.

The conclusion is reached that L-galactose-6-sulphate is probably a major unit in the structure of porphyran. This recalls the speculations of Jones and Peat[21], to which attention has recently been directed by Hirst[1]. Jones and Peat pointed out that a simple intramolecular oxidation-reduction reaction would convert D-galactose-1-sulphate into L-galactose-6-sulphate, which could then be converted by base-catalysed sulphate elimination into 3,6-anhydro-L-galactose. Hirst has added that it is also possible to postulate a mechanism of this type for the biosynthesis of L-guluronic acid in algin.

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BIOCHEMISTRY

Localization of Arginase in Rabbit Skin

ROGERS[1] has reported the induction of arginase in rabbit epithelium by the Shope papilloma virus. This conclusion was based on his findings of: (*a*) an increased uptake of arginine in papilloma tissue as compared to normal and hyperplastic whole skin; (*b*) the presence of appreciable quantities of arginase activity in papilloma tissue as compared to only trace amounts in extracts of normal skin.

We would like to propose an alternate interpretation of these findings on the basis of other data of arginase activity found in isolated tissues of the skin, interpreted in the light of the basic anatomy of the skin.

It is important to remember that the skin, exclusive of subcutaneous tissues, consists mainly of epithelium and connective tissue. The epithelial tissues, (epidermis, pilosebaceous follicles, and eccrine sweat units) constitute only a relatively small fraction of whole skin, the greater bulk being connective tissue (corium). Although arginase activity is detectable in whole skin[2,3], its localization is confined to the epidermis[4-7] and epidermal appendages[8]. It is not present in the corium, at least in amounts measurable by usual methods. In pathological conditions of human skin where the epidermis is both hyperplastic and hyperkeratotic (for example, psoriasis, verruca vulgaris, plantar callus), arginase activity in the epidermis is markedly increased[5,6]. In basal cell carcinoma, however, where the epidermis is hyperplastic but not hyperkeratotic, arginase activity has been found to be normal[6]. Activity of the enzyme in skin, therefore, seems to parallel the degree of epidermal keratinization.

The present work was performed to verify that the pattern of arginase activity in rabbit skin is similar to that found in the skin of man and other animals.

The trunk skin of a normal New Zealand albino rabbit was removed and cut into half-inch strips. The strips of skin were stretched tightly on a board with dissecting needles and the epidermis scraped from the underlying corium with a scalpel[9]. The removed epidermis contained no corium, although it was visibly contaminated with hair stubble. This material was lyophilized, defatted with ether, and ground in a Wiley mill fitted with a 60-mesh screen. Only 20 mgm. of dry, powdered epidermal material was obtained from the greater portion of the trunk skin. A specimen of whole skin (containing epidermis, epidermal appendages, and corium) was powdered in similar fashion, yielding several grams of material.

Arginase activity of each tissue specimen was microassayed as described previously[8]. In this assay approximately 1-mgm. samples of epidermis, weighed on a Kahn electrobalance, were used; 5-mgm. samples of whole skin were used. The enzyme was activated by incubating the tissue with 0·2 ml. of 0·05 M manganese chloride at 50° C. for 1 hr. Immediately thereafter 0·05 ml. of substrate, 1·7 M arginine (*p*H 9·5), was added and the suspension incubated for 1 hr. at 37° C. The reaction was stopped by the addition of 0·05 ml. of 1·2 M sulphuric acid. The urea formed was enzymatically hydrolysed by urease at *p*H 5·0; the ammonia was liberated from solution with saturated potassium carbonate and diffused to acid in the centre well of a Conway dish. The ammonia was Nesslerized and quantitated with a Beckman *DU* spectrophotometer at a wave-length of 420 mμ. The results are shown in Table 1.

Table 1. ARGINASE ACTIVITY IN RABBIT SKIN

Experiment No.*	Wt. sample (mgm.)	γ Urea nitrogen found after enzymatic reactions			Activity†
		Complete system	Boiled tissue system	Corrected	
Epidermis					
1	0·854	53 4	14·7	38·7	4,580
2	0 982	50 1	13 6	36 5	3,715
Whole skin					
3	5 3	84 5	41 7	42 8	790

* The results of each experiment are averaged values from two or more determinations.

† Expressed as milligrams urea nitrogen per 100 gm. dry, defatted tissue resulting from hydrolysis of arginine on incubation for 1 hr. at 37° C.

The values of arginase activity obtained indicate that the enzyme in rabbit skin is largely localized in the epidermis, and that the activity in whole skin is due to the epidermis and epidermal appendages thereof. Localization of arginase in rabbit skin is thus similar to that in the skin of man. In view of the fact that arginase activity increases in hyperplastic, hyperkeratotic human epidermis, it seems reasonable to expect a similar increase in papillomas of rabbit skin, which are also characterized by epidermal hyperplasia and hyperkeratosis. The conclusion that the Shope virus induces arginase in rabbit epidermis, therefore, seems unwarranted.

The significance of arginase in normal epidermis, as well as its elevated activity in hyperkeratotic epidermis, is nevertheless unexplained. Examination of normal human epidermis for the enzymes of the Krebs–Henseleit urea cycle has revealed that, of these enzymes, only arginase is present[10]. Thus, the role of arginase in epidermis does not seem to be the same as that classically ascribed to it in liver.

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Multi-stranded Deoxyribonucleic Acid as determined by X-Irradiation

IT has been widely assumed that DNA from all sources is two-stranded. However, much of the available data are consistent with more complex structures. We wish to emphasize that only those methods which actually count the number of strands per molecule are valid for examining this question. Enzyme kinetics is such a method, and has been used to show that calf thymus DNA is approximately two-stranded[1]. We have recently carried out a detailed investigation[2] of deoxyribonuclease II kinetics, using as substrates DNA samples from a variety of sources. Two classes of DNA molecules were found : namely, unitary molecules (two-stranded) and biunial molecules (four-stranded). The existence of four-stranded molecules, or pairs of double helices, has been confirmed by electron microscopy[3]. New evidence is presented here in support of these findings.

The decay in molecular weight brought about by X-irradiation has been examined as a function of X-ray dose. The following equation, which has been derived elsewhere[2], was used to express the results :

$$\frac{1-R}{R} = m(\Pi B)p^n = m(\Pi B)(kD)^n = [m(\Pi B)k^n](D)^n$$

where R is the ratio of the weight-average molecular weight after a given dose to the initial weight-average molecular weight ; $(1-R)/R$ is the number of molecular cleavages caused by the given dose ; m is the number of internucleotide bonds per polynucleotide strand ; ΠB is a function which takes into account the possibility that molecular cleavage can occur even though the single-chain scissions are not directly opposite one another ; p is the cumulative probability that a given bond will be cleaved (in the present communication, p is proportional to X-ray dose, D) ; n is the number of polynucleotide strands per molecule. When log $[(1-R)/R]$ is plotted against log D, as in Fig. 1, the slope of the resulting line is n.

This equation was derived for random degradation ; it has been shown[4] that use of the equation when degradation is non-random leads to apparent values of n which are too low. The question is whether or not X-rays produce random degradation. It has been shown[4] that the X-ray degradation of DNA in solution is brought about indirectly by the free radicals formed in water. These occur in clusters having an initial diameter of about 20 Å. and containing about five pairs of radicals. Thus, any clusters formed in the immediate vicinity of a molecule could produce several simultaneous strand cleavages in the same region. A certain degree of non-randomness,

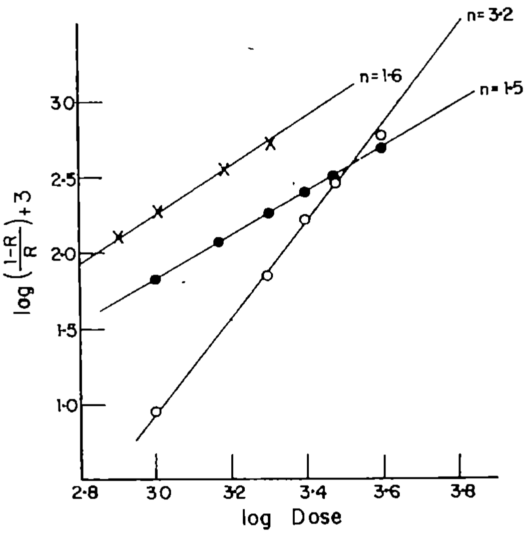

Fig. 1. Plot of log[molecular cleavages] versus log[dose], where the dose is given in rads. The slopes of the lines give values of n of 1 5, 1·6 and 3 2 for calf thymus, sea urchin sperm and *Pneumococcus* DNA respectively. The DNA samples studied have been designated as *OS*, *TS*-I and *LC*-II, respectively ; their initial molecular weights were 1·9, 2 2 and 1·6 million. The isolation and disaggregation of these samples are described elsewhere (ref. 5)

resulting in low values for the number of strands per molecule, is therefore to be expected.

Molecular weights were determined by light scattering. X-irradiation was carried out with a 180-keV. source, using a copper–aluminium filter, at a rate of 80 rads/min. The concentration of DNA was 0·1 mgm./ml., in 0·002 *M* sodium acetate. Solutions were made 0·2 *M* in sodium acetate immediately after irradiation and shaken with chloroform–octanol[5] before making light-scattering measurements, which were performed within 1–2 hr. Apparently no after-effects occurred under these conditions, since there was no change in molecular weight with time (up to about 24 hr.).

Fig. 1 shows that the number of strands (n) per molecule of DNA from calf thymus, sea urchin sperm and *Pneumococcus* appears to be 1·5, 1·6 and 3·2 respectively. For calf thymus and *Pneumococcus* DNA, n was previously found[2] by deoxyribonuclease II kinetics to be 1·7 and 4·2, ± 0·3, respectively, and sea urchin sperm DNA was predicted to be two-stranded[6]. The somewhat lower values obtained in the present investigation must be attributed to non-random attacks, and thus constitute minimum estimates; the previous work was shown to involve only random enzymatic attacks. The salient point is that n for *Pneumococcus* DNA is twice that for the other samples, all of which were undenatured and had the physical properties of stiff chains or double helices. Thus, the results of the present work support the conclusions reached earlier on the basis of enzyme kinetics and other evidence, that DNA molecules from non-proliferating sources are double helices, whereas molecules from proliferating sources consist of linked pairs of double helices.

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Interference in the Diphenylamine Procedure for estimating Deoxyribonucleic Acid

THE importance of deoxyribonucleic acid (DNA) in the control of cellular propagation is now well established. The amount in each somatic cell is constant for a particular species, deviations occurring with multinucleate cells or with polyploidy. With certain exceptions the DNA content of a tissue sample is directly related to the number of cells in that sample. This constancy has led to the widespread use of DNA as a standard of reference in biochemical investigations.

A general procedure for estimating DNA in tissue samples is the diphenylamine reaction; the variables of this reaction have been reviewed[1]. In most normal tissues this reaction satisfies the accepted criteria for a reliable assay method. The usual procedure is to extract the tissue with hot trichloracetic acid[2] and then to boil the extract with diphenylamine reagent for 5–15 min.; the resulting purple-blue colour is measured at 595 or 600 mμ. The extinction produced on boiling pure DNA with diphenylamine reagent increases rapidly with the time of boiling up to about 5 min., and then only slowly between 5 and 15 min.

Under certain conditions, the diphenylamine reaction is open to gross interference[1]; for example, the vivid green coloration produced by some plant extracts[3]. Dische[1] has proposed a multi-chromatic method for correcting such interference when the interfering chromagen has a sufficiently different spectrum from that of the colour produced by DNA. In general, however, such interferences are only specifically looked for when the visual appearance of the final diphenylamine colour is very different from the purple-blue of the DNA colour. The fact that interference may be considerable without appreciably affecting the visual appearance of the final colour, and the variation of such interference with time of boiling, do not appear to be sufficiently widely known.

Hot trichloracetic acid extraction of many tissues (and blood) solubilizes components other than DNA; these produce similar bluish colorations under the conditions of the diphenylamine reaction[4]. It is probable that these components are largely mucosaccharide in character[5]. After boiling with diphenylamine, the interfering chromagens have a main absorption maximum at 530 mμ and a smaller maximum at approximately 660 mμ (the exact position depends not only on the tissue but also on the time of boiling); these interfering colours develop more slowly on boiling with diphenylamine than is the case with DNA (ref. 4 and unpublished work). With many normal rat tissues, the amount of interference present is insufficient to produce appreciable error in estimations of DNA, providing the time of boiling is not longer than 10 min.; the extent of the interference increases rapidly with times of boiling in excess of 15 min.

With certain other tissues, however, there is considerably more interfering substance present, which produces a significant inaccuracy in estimations of DNA even when the time of boiling is 10 min. Fig. 1 shows the absorption spectra obtained by boiling a trichloracetic acid extract of involuting rat mammary gland with diphenylamine reagent for 4, 10 and 30 min. It can be seen that as the time of boiling is increased, the 530 mμ peak becomes more prominent; at the same time the extinction at 600 mμ also increases, unlike the behaviour of DNA itself. To assay DNA with any accuracy in such a tissue the time of boiling should not exceed 5 min. There is appreciably less interference present in mammary tissue at other stages in the lactation-cycle; the increased amount in involution appears to be largely the result of the accumulation of milk in the gland at this stage. The visual appearance of the final colour,

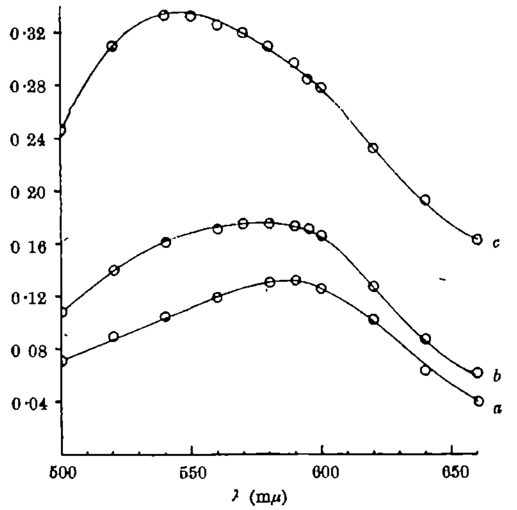

Fig. 1. Absorption spectra obtained after boiling trichloracetic acid extracts of rat mammary gland with diphenylamine reagent for varying times. Curves *a*, *b* and *c* obtained after boiling for 4, 10 and 30 min. respectively. The animal was in the first day of mammary involution, that is, the pups had been removed 24 hr. previously, at which time the rat had been in full lactation

even when considerable interference is present (as shown by a high 530-mμ peak), is not noticeably different from the colour produced by pure DNA.

Normal blood serum contains substances which interfere in the diphenylamine reaction for DNA by producing a peak at 530 mμ on boiling for long periods[3,7]. The concentration is raised in certain disease states, for example, inflammatory rheumatoid arthritis[4,6] and certain types of malignancy[7].

It is the purpose of this communication to point out that when the diphenylamine reaction is to be used for estimating DNA in tissues other than those already characterized (see refs. 1 and 2), or in which the diphenylamine reaction has been found to give results in agreement with alternative methods of assay, the variation in the absorption spectrum with time of boiling should be investigated to check the magnitude of possible interference with the time of boiling to be used. From the work on mammary gland and serum, it would appear that interference in the diphenylamine reaction can vary with the physiological status of the animal; care should therefore be exercised when using the diphenylamine reaction to compare DNA values under different physiological states when the concentration of interfering substance (mucosaccharide ?) may vary considerably.

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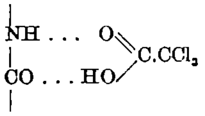
Binding of Trichloroacetic Acid by Protein

TRICHLOROACETIC acid (TCA) is widely used as a protein coagulant in the preparation of protein-free sera; but there is little information concerning its mode of action. Rowland[1] has shown that the complete separation of protein from milk requires a concentration of 8 per cent TCA at room temperature. We have determined the amounts of TCA bound by milk proteins at this concentration (Table 1).

Table 1. BINDING OF TRICHLOROACETIC ACID BY MILK PROTEINS

Protein	No. of samples examined	Trichloroacetic acid bound		
		Range	Average	
		gm. TCA/ 100 gm. protein	gm TCA/ 100 gm. protein	moles TCA/ 10^5 gm. protein
Casein	8	39–47	42	257
β-Lactoglobulin	2	92–102	97	594
Total whey protein	4	70–83	77	—

In 10^5 gm of whole casein[2] and β-lactoglobulin[2] there are 107 and 120 cationic side-chain groups respectively. If all these were available for binding TCA there remains a considerable number of sites at which the acid must be bound to account for the figures quoted above. We consider that these sites are provided by peptide bonds, the TCA being held in the following manner:



The elimination of bound water and its replacement by the strongly non-polar $Cl_3C.C$— radical destroys the solubility of the protein in water and tends to render it soluble in non-polar solvents. TCA–protein complexes dissolve to a varying degree in absolute alcohol or acetone, and the soluble fraction can be transferred to benzene and chloroform[3]. The alcohol-solubility of the TCA–protein complex varies over a narrow range for any particular undenatured protein and is related to the degree of binding of TCA by the protein (Table 2).

Table 2. SOLUBILITY IN ABSOLUTE ALCOHOL OF THE VARIOUS MILK PROTEINS AFTER PRECIPITATION WITH TCA

Protein	No. of samples examined	Final concentration of TCA	Solubility in alcohol	
			Range (per cent)	Average (per cent)
Casein	14	0·5 *M*	2 87– 5·38	3·95
β-Lactoglobulin	4	,,	69 0 –75·0	72·0
α-Lactalbumin	2	,,	36 0 –41·0	39·0
Globulins	1	,,	10·0	10 0
Proteose	1	,,	9 0	9·0

In the case of TCA–casein the alcohol-solubility is unaffected by particle size and the fraction dissolved contains all the known casein fractions in approximately the same ratio as they are present in native casein. A reduction in the number of TCA binding sites reduces the alcohol-solubility of the TCA–protein, and we have used this index of solubility to follow protein denaturation, protein–protein interaction and protein–sugar interaction in both model systems and in heated milk.

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Diglyceride Kinase and Phosphatidic Acid Phosphatase in Erythrocyte Membranes

WE have recently provided evidence that in the avian salt gland sodium is pumped by a mechanism which we have termed the phosphatidic acid-cycle[1,2]. In this mechanism phosphatidate functions as a sodium ion carrier in the membrane; the phosphatidic acid-cycle consists of two enzymes—diglyceride kinase, which forms phosphatidate from diglyceride and adenosine triphosphate, and phosphatidic acid phosphatase, which hydrolyses phosphatidate to yield diglyceride and orthophosphate. If the phosphatidic acid-cycle is a general mechanism for active sodium transport one would expect to find the enzymes of this cycle in all membranes which actively pump sodium. This preliminary report is concerned with the presence of these enzymes in erythrocyte membranes.

Membranes were prepared from hæmolysed human erythrocytes[3] and were incubated with an adenosine triphosphate-γ-phosphorus-32 generating system[4]. Under these conditions, phosphatidic acid became labelled with phosphorus-32; no other radioactive phosphatides were seen on chromatograms of the total lipid extracts. The radioactivity found in the phosphatidic acid spot on the chromatograms accounted for 80–100 per cent (uncorrected for breakdown of phosphatidic acid during chromatography) of the total radioactivity in the lipid extract (Table 1). These results indicate that phosphatidic acid is formed from adenosine triphosphate in the erythrocyte membrane.

Addition of diolein to the incubation system markedly increased the formation of phosphatidic acid; this indicates the presence of diglyceride kinase in the erythrocyte membrane. In the presence of added diglyceride the diglyceride kinase was stimulated by potassium, the optimum potassium concentration being 60–80 m.equiv./l. Sodium was on average about one-fifth as effective as potassium. No stimulation by sodium was observed in the presence of optimum concentrations of potassium.

Table 1. DIGLYCERIDE KINASE IN ERYTHROCYTE MEMBRANES

Exp. No.	Cation concentration (m equiv./l)		Diolein added	Radioactivity in phosphatides (counts/min./mgm. dry weight of ghosts)	
	Sodium	Potassium		Total extract	Phosphatidic acid
1	60	30	0	375	298
			10 μl.	1,420	1,400
2	0	0	10 μl.	667	
	0	20	10 μl.	820	
	0	40	10 μl.	951	
	0	80	10 μl.	1,180	
	0	120	10 μl.	1,180	
	16 5	0	10 μl.	835	
	31	0	10 μl.	920	
	62	0	10 μl.	945	
	93	0	10 μl.	910	

1 2 mgm. (dry weight) of erythrocyte ghosts were incubated in 0 25 ml. of incubation medium for 1 hr. at 37° C. After incubation the radioactivity of the total lipid extract and phosphatidic acid were determined as described previously (ref. 4). The incubation medium consisted of the following (expressed as final concentrations): imidazole–histidine–*tris* buffer, *p*H 7·1 (0·025 *M*), magnesium sulphate (0 002 *M*), *tris* carbamyl phosphate-^{32}P (0 0035 *M*), dialysed carbamate kinase from *S. faecalis* (0 3 units/ml.), *tris* fluoride (0 01 *M*), *tris* adenosine diphosphate (0·0004 *M*). The specific activity of the carbamyl phosphate-^{32}P was 2 5 × 10^6 counts/min./μmole.

Incubation of erythrocyte membranes with phosphatidic acid yielded orthophosphate (Table 2), indicating the presence of phosphatidic acid phosphatase. Two types of phosphatase activity were present in the membrane, one of which was independent of the presence of magnesium and one which was dependent on the presence of magnesium. The optimum concentration of magnesium was about 1 m.equiv./l. Sodium ions inhibited somewhat the magnesium-independent phosphatase but stimulated the magnesium-dependent phosphatase. In some experiments the magnesium effect was seen only in the presence of sodium, but in others a partial stimulation by magnesium occurred in the absence of sodium. Preliminary experiments indicate that the dependence of the magnesium effect on the presence of sodium develops with ageing of the preparation.

Table 2. PHOSPHATIDIC ACID PHOSPHATASE IN ERYTHROCYTE MEMBRANES

Exp. No.	Cations added*	Total phosphatidic acid phosphatase (mμmoles of ortho-P formed per mgm. dry wt. of ghosts)	Mg^{++}-dependent phosphatidic acid phosphatase (mμmoles of ortho-P formed per mgm. dry weight of ghosts)
1	None	5·1	
	Mg^{++}	5·1	0
	Na^+	3·1	
	$Na^+ + Mg^{++}$	9·1	6·0
	Li^+	4 9	
	$Li^+ + Mg^{++}$	9·2	4 3
	NH_4^+	4·1	
	$NH_4^+ + Mg^{++}$	6 1	2 0
	K^+	5·1	
	$K^+ + Mg^{++}$	6·2	1 1
	Cs^+	5 3	
	$Cs^+ + Mg^{++}$	5 2	0
2	None	4 6	
	Mg^{++}	9 1	4 5
	Na^+	4·0	
	$Na^+ + Mg^{++}$	13 2	9·2

* The final concentration of magnesium was 0·8 m.equiv./l. and the final concentration of each of the monovalent cations listed was 155 m.equiv./l.

5·5 mgm. (dry weight) of erythrocyte ghosts were incubated in 1·0 ml of incubation medium at 37° C. for 1 hr. The incubation medium contained the following (expressed as final concentration): *tris* dioleyl phosphatidate-^{32}P (0·002 *M* in experiment 1 and 0·003 *M* in experiment 2) and imidazole–histidine–*tris* buffer, *p*H 7 1 (0 03 *M*). The *tris* phosphatidate ranged in specific activity from about 10 counts/min./mμmole to about 120 counts/min./mμmole After incubation 0·05 ml of 10 per cent crystalline bovine albumin was added to the incubation vessel, followed by 1 ml. of 10 per cent trichloracetic acid. After centrifugation the trichloracetic acid extract was washed three times with wet ether, and a 1-ml. aliquot of aqueous phase was dried on a planchet and counted. An incubation with boiled ghosts was used as the blank. All values are averages of triplicate incubations

The combined actions of diglyceride kinase and phosphatidic acid phosphatase results not only in the synthesis and breakdown of phosphatidic acid but also in adenosine triphosphatase activity. Adenosine triphosphatase activity has been shown to be closely related to the action of the sodium–potassium pumps in both nerve[5] and erythrocyte[3] membranes. This adenosine triphosphatase is dependent on the presence of both sodium and potassium and also requires magnesium. Since the combined activities of the two enzymes of the phosphatidic acid-cycle require sodium, potassium and magnesium, it is possible that this cycle may be identical with the adenosine triphosphatase activity related to the sodium–potassium pumps.

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Lecithin-splitting Enzyme of *Vibrio* El Tor

IT was known that *Vibrio cholerae* and *Vibrio* El Tor excrete a lecithin-splitting enzyme in their culture medium[1-3], but the nature of this enzyme was not properly ascertained. The present communication is concerned with the characterization of the enzyme excreted by *Vibrio* El Tor with special reference to some of its important properties. The enzyme was isolated from the culture filtrate of the organism, which was grown aerobically in surface cultures using peptone–water medium for 48 hr. The culture filtrate was fractionated by ammonium sulphate and the precipitate obtained between 50–75 per cent saturation contained all the enzyme activity. This was dissolved in 0·01 *M* phosphate buffer and after dialysis against the same buffer was kept frozen at − 15° C. for about four weeks, up to which time no loss of enzyme activity has been noticed. Addition of glycerol is not necessary for its storage.

The enzyme has been found to attack only lysolecithin molecules liberating acid-soluble phosphorus and acid-labile choline compounds without any liberation of free choline and is inactive towards purified lecithin. The incubations were performed in 10-ml. stoppered centrifuge tubes into which the requisite amount of lipid substrate in chloroform solution (usually 0·75 μmole) was added. The solvent was removed *in vacuo* at about 40° C. and the lipid was then mixed up in buffer solution (50 μmoles barbital buffer of *p*H 8·0, which has been found to be its optimum *p*H). The enzyme equivalent to approximately 0·15 mgm. protein was then added and the total volume of the incubation mixture was kept 1 ml. The incubations were made at 37° C. for 1 hr., after which the reaction was stopped by the addition of trichloracetic acid solution to a final concentration of 5 per cent in the presence of bovine serum albumin (5 mgm. per flask). This was centrifuged, the centrifugate was extracted once with three volumes of a mixture of chloroform and *iso*butanol (2 : 1, v/v) and was again centrifuged. The aqueous portion which was separated was analysed for the reaction products. It has been noted that there is a decrease in the fatty acyl ester group and simultaneous increase in acid-soluble phosphorus, and these are in quantitative proportion with the release of acid-labile choline compound. This choline compound has been identified as glycerophosphoryl–choline with the help of paper chromatography according to the method described by Dawson and Rowlands[4]. The enzyme is therefore characterized as 'phospholipase *B*'.

Unlike any other phospholipase *B* from *Penicillium notatum*[5], *Aspergillus niger*[6] or from any animal tissue such as pancreas[7] and liver[8], this enzyme has a *p*H optimum of 8·0 and is fairly stable towards heat denaturation. It is destroyed only on heating at 100° C. for 15 min., and heating for 5 min. at that temperature causes about only 50 per cent inactivation. It is fairly insensitive towards metal ions, and when used at a final 1 m*M* concentration only zinc causes about 40 per cent inhibition, whereas copper, manganese, calcium and magnesium have practically no effect. Of the inhibitors studied at 10^{-3} *M* final concentration, cyanide, azaguanine and chloramphenicol have no effect, azide and dinitrophenol inhibit the enzyme to the extent of about 15–20 per cent, whereas sodium fluoride, versene and *p*-chloromercuribenzoate individually cause about 45 per cent inhibition. The inhibition by versene and by *p*-chloromercuribenzoate can be fully reversed by the addition of calcium and glutathione respectively. It has further been observed that glutathione and also cysteine bring about some stimulation of the enzyme. Work on the properties and also on the mechanism of excretion of this enzyme by both *Vibrio cholerae* and *Vibrio* El Tor is in progress.

We wish to thank Prof. B. C. Guha for his advice and interest in this work, and also Dr. R. M. C. Dawson, Cambridge, England, for his gift of a sample of glycerophosphoryl choline.

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Immunity of Bee Keepers to Some Constituents of Bee Venom : Phospholipase-*A* Antibodies

FOR many years bee venom has been used in the treatment of arthritis and rheumatism, but with little evidence of its value[1]. Bee venom contains three important constituents : the main toxic fraction I, and the enzyme fraction II, containing phospholipase and hyaluronidase. Each enzyme has been isolated in pure form[2,3]. The hæmolytic effect of the venom on blood corpuscles is caused directly by the toxic fraction I, and indirectly by the phospholipase-*A* activity[4]. Among other effects of phospholipase-*A* is retardation of egg yolk coagulation[5].

Antibodies against the toxic fraction have not been found ; those against the enzymatic part of the venom have been demonstrated in the sera of mammals injected with bee venom for a long time[6,7]. We have therefore examined the sera of bee keepers to ascertain whether they contain antiphospholipase bodies.

Samples of blood from bee keepers were taken as test samples, and from ordinary men as control samples. The blood was left to clot at room temperature, then placed in a refrigerator overnight. Serum, obtained by centrifuging and decanting, was kept in a refrigerator until required. Natural bee venom was dissolved in physiological saline (0·85 per cent) in concentrations of 1 : 1,000, 1 : 2,000 and so on up to 1 : 64,000.

To 0·05 ml. bee keeper serum were added 0·05 ml venom solution and, after at least 15 min. at room temperature, 2 ml. of egg yolk diluted with physiological saline in the proportion of 4 : 6. The mixture was shaken, heated for 1 hr. in a water-bath at 37° C., and the tubes then plunged into boiling water and the time of coagulation recorded with a stop watch. The emulsion was considered to coagulate when no traces of the egg yolk adhere to a thick glass rod inserted repeatedly into the tubes containing the mixture. Two control experiments were carried out in the same way, one with normal serum and the other with saline instead of serum.

The methods of calculation were as follows:

Venom end concentration: 0·05 ml. venom was added to 0·05 ml. serum and 2·0 ml. egg yolk; the final dilution was 0·05 : 2·1 = 1 : 42. When original venom concentration is 1 : 2,000, the end concentration is 1 : 84,000.

Serum end concentration: 0·05 ml. serum was added to 0·05 ml. venom and 2·0 ml. egg yolk; the final dilution was 0·05 : 2·1 = 1 : 42. Serum itself was used without original dilution, therefore its end concentration is the same as the final dilution = 1 : 42.

Serum in ml. : Venom in gm.: 1 : 42/1 : 84,000 = 2,000 : 1.

Effect of serum of normal men: This is considered as activity if the time of coagulation is more prolonged, and as inhibition if time is shorter, than time of control; for example, average control saline = 24·8, average control serum of normal men = 26·3; effect of normal serum

$$= \frac{26 \cdot 3 - 24 \cdot 8}{24 \cdot 8} \times 100$$

= 6 per cent activation.

Effect of serum of bee keepers: The effect was related to the effect of serum of normal men; for example, average control serum of normal men = 26·3, average test serum of bee keepers = 21·6; effect of bee keepers serum

$$= \frac{26 \cdot 3 - 21 \cdot 6}{26 \cdot 3} \times 100 = 18$$

per cent inhibition.

The results obtained in a series of experiments are summarized in Table 1.

Table 1. Effect of Sera from Bee keepers and Control on the Coagulation-time of Egg-yolk

	Venom original concentration	Control saline	Control serum	Test serum
	1 : 2,000			
Mean coagulation time (min.)		24·8	26·3	21·6
Standard error of mean		4·28	4·88	4·56
	1 : 4,000			
Mean coagulation time (min.)		9·8	10·1	9·7
Standard error of mean		0·4	0·26	0·88
	1 : 8,000			
Mean coagulation time (min.)		4·8	4·9	3·0
Standard error of mean		0·57	0·30	0·41
	1 : 16,000			
Mean coagulation time (min.)		2·7	2·0	1·7
Standard error of mean		0·41	0·28	0·16

The above figures represent the averages for 4 saline controls, 6 normal sera controls and 10 bee keepers sera.

From Tables 1 and 2, it is clear that normal serum has no antiphospholipase effect against high final concentration of venom (1 : 2,000, 1 : 4,000 or 1 : 8,000). In the first two concentrations the activity of the enzyme was increased. Serum from bee keepers showed an inhibitory effect on the enzyme at previous concentrations; it reached more than 40 per cent in concentrations of 1 gm. venom to 4 litres serum.

Table 2 shows that phospholipase-*A* of bee venom was activated by serum of normal men when the concentration of the latter to that of venom was 2,000 : 4,000 : 1, and 8,000 : 1. Very dilute concentrations of 16,000 : 1 were inhibited by normal serum. Serum of bee keepers inhibited the phospholipase-*A* effect in all concentrations used.

Table 2. Quantitative Comparison of Inhibition of Phospholipase-*A* Activity by Sera of Normal Men and Bee keepers

Venom end concentration	Serum end concentration	Serum : Venom (ml. : gm.)	Effect of serum of normal men	Effect of serum of bee keepers
1 : 84,000	1 : 42	2,000 : 1	activation, 6 per cent	inhibition, 18 per cent
1 : 168,000	1 : 42	4,000 : 1	activation, 3 per cent	inhibition, 4 per cent
1 : 336,000	1 : 42	8,000 : 1	activation, 2 per cent	inhibition, 39 per cent
1 : 672,000	1 : 42	16,000 : 1	inhibition, 25 per cent	inhibition, 15 per cent

In low concentrations of venom, such as 1 : 16,000, both normal serum and bee keepers' serum showed an inhibition of phospholipase-*A*; the latter being about 10 per cent more inhibitory.

We thank the Council for Combined Units for facilities in obtaining blood samples from bee keepers working in its apiaries; Dr. K. A. Forster, for providing carefully dried bee venom prepared by Mack Company, Illertissen, Germany; Blood Bank of Ain Shams University, for samples of normal blood of different blood groups; and Dr. M. Shabrawi for useful help.

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PHYSIOLOGY

Effect of Urinary *p*H on 5-Hydroxytryptamine Excretion in the Rat

Urinary *p*H influences the excretion-rate of some weak acids and bases[1,2], a more rapid excretion of weak acids occurring in alkaline urine and of weak bases in acid urine. Most of the known compounds which behave in this way are not found naturally

Table 1. SHOWING URINARY EXCRETION OF 5-HYDROXYTRYPTAMINE AND 5-HYDROXYINDOLEACETIC ACID AS PERCENTAGE OF DOSE OF L-^{14}C-5-HYDROXYTRYPTOPHAN OR ^{14}C-5-HYDROXYTRYPTAMINE IN 'ACID' OR 'ALKALINE' RATS

Hr. of exp.	DL-^{14}C-5-Hydroxytryptophan administration															
	Exp. 1				Exp. 2				Exp. 3				Exp. 4			
	Alkaline		Acid		Alkaline		Acid		Alkaline		Acid		Alkaline		Acid	
	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA
1	0·05	0·2	0·3	0·1	0	0	1·6	0	0·25	0	0·05	0·05	1·6	0·05	3·05	0·2
2	0·3	0·3	0·6	0·05	0·6	0·4	3·2	0·2	0·2	0·05	1·3	0·4	1·4	0·4	2·4	0·25
3					0·6	0·2	1·6	0·05	0·1	1·0	0·4	1·4	1·6	0·4	0·8	0·2
4									0·05	2·0	0·05	0·6	0·9	0·2	0·5	0
5													0·9	0	0·3	0
6																
Total (per cent)	0·35	0·5	0·9	0·15	1·2	0·6	6·4	0·25	0·6	3·05	1·8	2·45	6·4	1·05	7·05	0·65

Hr. of exp.	DL-^{14}C-5-Hydroxytryptophan administration				^{14}C-5-Hydroxytryptamine administration											
	Exp. 5				Exp. 6				Exp. 7				Exp. 8			
	Alkaline		Acid		Alkaline		Acid		Alkaline		Acid		Alkaline		Acid	
	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA	5HT	5HIAA
1	3·0	0·55	2·1	0·4	0·05	0·2	0·2	0	0·2	0·35	0·2	0·75	0·85	0·9	0·65	0·35
2	2·7	4·8	4·2	0·8	0·2	0·7	0·15	0·35	0·05	0·6	0·1	1·1	0·4	2·85	1·3	7·15
3	2·2	1·8	4·2	0·6	0·05	4·2	0·1	2·4	0·05	0·7	0·1	0·5	0·3	1·45	0·7	2·1
4	1·5	0·6	1·85	0·4	0	3·5	0·1	0·35	0	0·25	0·05	0·2				
5	1·05	0·5	1·1	0·4	0	0·8	0	0·1	0	0·4	0·05	0·2				
6	0·3	0·2	0·45	0·5												
Total (per cent)	10·75	8·45	13·9	3·1	0·3	9·4	0·55	3·2	0·3	2·3	0·5	2·75	1·55	5·2	2·65	9·6

Female white Wistar rats of 200–250 gm. body-weight were depleted of sodium chloride by the dietary method of Milne *et al.*[4]. Two rats, matched for weight, were used in each experiment. One was injected intraperitoneally with 10 ml. isotonic sodium sulphate ('acid rat' – urine pH 5·5–6·0 throughout experimental period) and the other with 10 ml. isotonic sodium bicarbonate containing 10 mgm. acetazolamide ('alkaline rat' – urine pH 8·0–8·5 throughout experimental period). The injection solutions also contained 30 mgm. per kgm. body-weight of β-phenyl*iso*propylhydrazine ('Cavodil'). One hr. later an indwelling catheter was placed in position under pentobarbitone–ether anæsthesia which was continued throughout the experiment. After 1·5 μc. DL-^{14}C-5-hydroxytryptophan (exps. 1–5) or 1·5 μc. ^{14}C-5-hydroxytryptamine (exps. 6–8) had been injected intraperitoneally, hourly collections of urine were made. Bladder washings of 3 × 1 ml. isotonic sodium chloride, obtained at the end of each hour, were added to urine excreted during that hour.

Specimens of urine were analysed for total number of counts and portions were also extracted (refs. 5, 6) and assayed for ^{14}C-labelled 5-hydroxytryptamine or 5-hydroxyindoleacetic acid using a thin end-window Geiger counter.

in the body. The only endogenous substances so far described, the excretion-rate of which is dependent on urinary pH, are ammonia[3] and indoleacetic acid[4]. We present evidence here to show that the rate of excretion of 5-hydroxytryptamine (5HT) is similarly influenced by pH, being higher in acid urine (Table 1).

Data from man indicate that 5HT is normally present in the urine in low concentration[7], although larger amounts are found after parenteral administration of 5-hydroxytryptophan (5HTP)[8] and in patients with certain tumours[9]. The level probably depends to an appreciable extent on the amount of 5HTP presented to the kidney for direct renal decarboxylation. In rats used in experiments described here, the rate of 5HT excreted was greater after 5HTP injection than after 5HT (Table 1). Presumably most of the free extra-renal 5HT was either rapidly metabolized or absorbed by platelets. In these animals monoamine oxidase had been blocked with β-phenyl*iso*propylhydrazine to prevent further renal metabolism of 5HT. The absolute amounts of 5HT labelled with carbon-14 used were probably insufficient to interfere with renal hæmodynamics.

It will be seen (Table 1) that there was a fairly wide variation in 5-hydroxyindole excretion between experimental groups, which may be accounted for, to some extent, by differences in such variables as depth of anæsthesia. Anæsthesia and environment of the pair of animals used in each single experiment were similar however, so that despite the between-group variation, the tendency of the 'acid rat' to excrete 5HT more rapidly than the 'alkaline rat' (Fig. 1) is probably significant.

After 5HTP administration, increased rate of 5HT excretion in the 'acid rat' is complemented by a decrease in 5-hydroxyindoleacetic acid (5HIAA) excretion and vice versa (Table 1). However, as 5HIAA is known to have an active transport mechan-

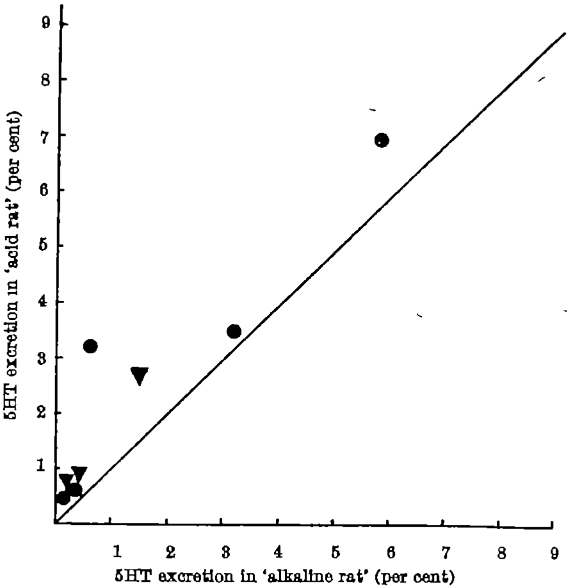

Fig. 1. Excretion of 5-hydroxytryptamine (5HT) in acid and alkaline urine expressed as percentage of total counts injected. ▲, 5HT administration; ●, 5HTP administration

ism for its renal elimination[4,10] these changes presumably reflect the absolute amounts of 5HT involved; if more is excreted by the kidney, less is available for conversion to 5HIAA. In experiments 6–8, however, in which 5HT itself was administered, the concentrations of urinary 5HT probably form too small a fraction of the total excretion of 5-hydroxyindole metabolites to produce any experimentally obvious reciprocal change in 5HIAA values. The mechanism of 5HIAA production under conditions of heavy monoamine oxidase blockade, which has been observed by many workers, is not understood.

From theoretical considerations[1], it is rather surprising that such a lipid-insoluble compound as 5HT[11] is excreted by a non-ionic diffusion mechanism. Elucidation of this problem must await further study. But one practical implication of the work that we have described is that investigations in which urinary 5HT measurements have been used as an index of 5HTP decarboxylase activity[12,13] will probably need re-evaluation.

We are grateful to Benger Laboratories, Ltd., for generous supplies of 'Cavodil'.

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Influence of Sodium on the Transport of Magnesium across the Intestinal Wall of the Rat *in vitro*

SINCE the first description of hypomagnesæmia in adult cattle by Sjollema and Seekles[1] there have been numerous investigations into the underlying causes of this condition. Herbage analysis of the pasture where the disease has occurred shows that it is not usually due to an absolute dietary deficiency, although Norwegian workers[2] have shown that this may occasionally be so. It has been suggested that the absorption of magnesium may be impaired when cattle or sheep are suddenly moved to rapidly growing young pastures. Head and Rook[3] have suggested that this may be due to the accumulation of ammonia in the rumen, and Kemp and 'T Hart[4] have provided evidence which has incriminated high levels of potassium in the pasture at this time.

In an attempt to find out more about the factors which influence the availability of magnesium to the animal, a series of experiments has been undertaken using an *in vitro* preparation of rat small intestine[5] in which oxygenated Krebs's bicarbonate saline[6] containing 0·5 per cent glucose is circulated through the intestinal lumen, and the water and solutes which are transported to the serosal surfaces are collected below. Magnesium has been estimated, using a slight modification of the method of Mann and Yoe[7], and sodium, using an EEL flame photometer.

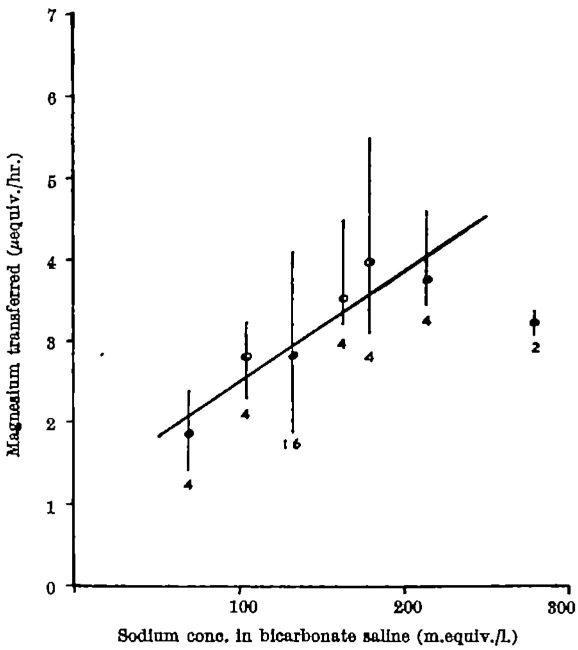

Fig. 1. Relation between magnesium transferred per hr. and sodium concentration in the bicarbonate saline. Vertical line shows range of results. Number below indicates number of experiments. Points are arithmetic means. ○, young rats; ●, old rats.

The amount of magnesium (μequiv./hr.) transported to the serosal surface was found to vary when the circulating concentration of sodium chloride was altered. This is shown graphically in Fig. 1, where the regression line for 36 readings has been drawn (magnesium transferred = 0·015 sodium concentration − 0 92; correlation coefficient $r = 0{\cdot}65$; $P < 0{\cdot}001$). The concentration of the other ions in the bicarbonate saline remained unaltered. The two experiments in which the concentration of sodium was highest (280 m.equiv./l., that is, an increase of 137 m.equiv./l. over Krebs's bicarbonate saline) have been omitted when the regression line was calculated. At this concentration the volume of water transported was reduced from a normal value of 2–7 ml. to less than 1 ml./hr., confirming the findings of Smyth and Taylor[5] on the effect of increasing osmotic pressure on such a preparation.

Two groups of rats were used in the experimental work, and it will be seen from Fig. 1 that the mean values for the younger group (about 12 weeks old) invariably fell above the regression line, but the means for the older group (about 20 weeks old) were below the line. This suggests that the younger group were the more efficient at transporting magnesium to the serosal surface.

The effects on magnesium transport of the addition of ammonium chloride, and increasing the potassium concentration in the circulating solution (from a normal value of 5·9 m.equiv./l.) by the addition of potassium chloride, are shown in Table 1, and are compared with the results for Krebs bicarbonate saline. It will be seen that neither of these salts

Table 1

Concentration in bicarbonate saline (m.equiv./l.)	Magnesium transported (μequiv./hr.)	No. of experiments
K^+ – 27	2·97	3
K^+ – 40	2·72	2
K^+ – 84	2·72	1
NH_4^+ – 20	3·01	2
NH_4^+ – 100	2·82	1
Krebs's bicarbonate saline K^+ – 5·9 ; NH_4^+ – 0)	2·81	16

appears to influence the transport of magnesium under these conditions. The influence of sodium chloride on magnesium transport is therefore probably due to the sodium rather than to the chloride ion, since the chloride was common to all three salts.

It is interesting to note that the association between sodium and magnesium transport across the intestinal wall, reported here, is similar to that recently described for the human kidney[8], where the rate of excretion of magnesium was found to vary directly with the concurrent excretion of sodium chloride.

The relation of these findings to ruminant hypomagnesæmia is difficult to evaluate, since the conditions in this *in vitro* preparation of rat small intestine are very different from those of the ruminant gastrointestinal tract, although it does suggest that attention should be given to the influence of varying sodium-intake on magnesium absorption in the intact animal.

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Clinical Application of a Serological Method for the Determination of Human Chorionic Gonadotrophin

It was early observed that prolonged administration to animals of gonadotrophic hormone from human pregnancy urine was accompanied by gradually diminished effects[1]. This phenomenon has been found to be due to the formation of an anti-gonadotrophic factor demonstrable only by its capacity to impair the gonadotrophic reaction in rodents[2]. Previous to our earlier report[3], there had been no demonstration of the antigonadotrophic factor by serological *in vitro* methods. Precipitating and complement-fixing antibodies had been obtained by immunization of rabbits with gonadotrophic hormone prepared from human pregnancy urine[4,5]. These antibodies, however, were shown to be due to contaminating, antigenically active substances[6].

In contrast to these investigators, we have been able to demonstrate that human chorionic gonadotrophin can stimulate the production, demonstrable *in vitro*[3], of antibodies against itself. This was accomplished by the inoculation of a purified human chorionic gonadotrophin preparation (Gonadex-Leo, supplied by courtesy of Dr. Högberg, AB Leo, Halsingborg, Sweden) together with an adjuvant into a heterologous species (rabbit). The presence of human chorionic gonadotrophin antibodies in the rabbit sera was demonstrated with the complement fixation test. With these antisera, human chorionic gonadotrophin could then be determined in serum as well as in the urine of pregnant women by means of this serological test. Two other reports, in principle confirming our results, have since been published. These showed that the estimation of human chorionic gonadotrophin in urine was possible with the precipitin test[7] and the hæmagglutination inhibition reaction[8].

In further experiments we have been particularly interested in evaluating the sensitivity and specificity of our serological procedure for the determination of human chorionic gonadotrophin with a view to its clinical applicability. From practical as well as physiological considerations, we have preferred to analyse serum rather than urine.

Preparation of antisera. Twelve rabbits were immunized, each with 12,500 I.U. of human chorionic gonadotrophin in adjuvant (10 parts of the hormone in saline, 8·5 parts paraffin oil and 1·5 parts 'Arlacel *A*', the mixture containing 10 mgm. of finely powdered *Mycobacterium butyricum* per 100 ml.) administered in a total volume of 20 ml. on three occasions at intervals of three weeks. The mixture was given at four sites subcutaneously and intramuscularly. A booster injection of 3,000 I.U. of the hormone in saline was given intravenously four to six weeks after the last inoculation. Blood was withdrawn by cardiac puncture one week after the booster injection. Serum was separated, heated at 56° C. for 30 min. and stored at –20° C.

Serological technique and standardization of antisera. The rabbit sera were tested for the presence of human chorionic gonadotrophin antibodies in complement fixation tests against the hormone dissolved in sera from non-pregnant individuals. The tests were carried out as 'chess-board' titrations according to the plate technique of Fulton and Dumbell[9] as modified by Svedmyr, Enders and Holloway[10]. Twofold serial dilutions were employed with barbiturate buffer as diluent. Simultaneous complement titrations with antigens, antisera and diluent were included in each experiment to rule out false positive reactions due to anticomplementary effects.

All antisera were tested against sera from non-pregnant women. Most rabbit sera gave negative results. With a few antisera positive reactions in low-antiserum titres of the type described earlier were encountered[3]. These reactions could be completely overcome by means of a simple absorption procedure[3].

The antisera were then tested with a solution containing 1,500 I.U. of the hormone; its concentration was checked against the international standard. A typical 'chess-board' titration is shown in Table 1. The hormone solution diluted 1/6,400 still gives positive reactions, which implies that with this anti-

Table 1. 'CHESS-BOARD' TITRATION OF HUMAN CHORIONIC GONADOTROPHIN WITH SERUM FROM RABBIT IMMUNIZED WITH THE HORMONE

Antigen	Dilution of antigen	Dilution of antiserum 8	16	32	64	128	256	512
Human chorionic gonadotrophin 1,500 I.U. per ml.	50	+	+	+	+	−	−	−
	100	+	+	+	+	+	−	−
	200	+	+	+	+	+	+	−
	400	+	+	+	+	+	+	−
	800	+	+	+	+	+	+	−
	1,600	+	+	+	+	+	+	−
	3,200	−	+	+	+	+	+	−
	6,400	−	−	+	+	−	−	−
	12,800	−	−	−	−	−	−	−

serum 0·25 I.U. of human chorionic gonadotrophin per ml. can be determined. The maximal titres of the hormone solution varied from 3,200 to 12,800. From Table 1 it is apparent that antiserum diluted 1/32 is suitable for carrying out quantitative determinations of the hormone in a routine procedure. This has also been confirmed in 'chess-board' titrations with specimens from pregnant women.

Clinical investigations. Blood samples were collected from 194 patients. Serum was separated, heated at 56° C. for 30 min. and stored at −20° C. until examined. 148 women were pregnant. Pregnancy was diagnosed by gynæcological examination and in early cases by biological pregnancy tests[11]. (The biological pregnancy tests were performed at the Hormone Laboratory of the Department of Obstetrics and Gynæcology, Karolinska Sjukhuset.) As all samples were collected 3–7 months before testing, all cases were also checked by follow-up. With the serological determination procedure of chorionic gonadotrophin, all specimens from pregnant women gave positive results. No false positive reactions were found. Fig. 1 illustrates the quantitative results obtained with sera from pregnant women varying in gestational stage from 5 to 42 weeks. The titres vary from 2 to 1,024 corresponding to 0·7 to 340 I.U. of hormone per ml. serum. The highest titres were observed during the first trimester. High values were, however, also found late in pregnancy.

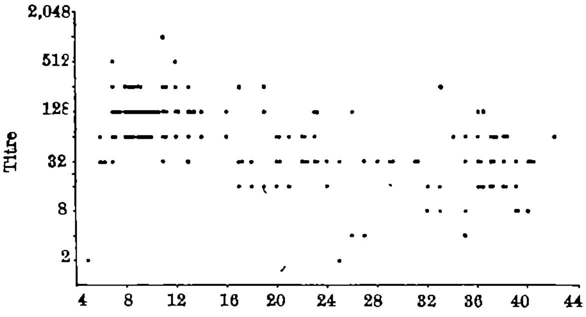

Fig. 1. Results of serological determination of human chorionic gonadotrophin in serum of 148 pregnant women. (According to standardization tests, a minimal amount of 0·35 I.U. of the hormone per ml. could be determined with the antiserum used)

These results indicate that the serological procedure presented here is a sensitive and specific method for the determination of human chorionic gonadotrophin. Its suitability for the diagnosis of pregnancy seems apparent. The fact that the test can be performed on serum increases the clinical importance of the quantitative data obtained. Work is now in progress on quantitative serological analyses of human chorionic gonadotrophin in serum in various pathological conditions. We are also planning simultaneous quantitative biological and serological determinations of the hormone.

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Subjective Analysis of Saccadic Eye Movements

IN the course of an investigation into the mechanics of eye movements, using mainly electrical recording methods[1], some of the findings have been checked by a simple subjective method. This enables an observer to examine his own saccades, which he sees traced out before him in the form of displacement-time curves.

The subject views, from a distance of about 50 cm., a cathode ray oscilloscope, on which is a straight horizontal line, generated by a time-base running at a velocity between 20 and 50 cm./sec. When small downward vertical saccades of a few degrees extent are made, a tracing of the type shown on the left of Fig. 1 is seen, and when upward vertical movements are made, the tracing is usually that shown on the right. An explanation of how the effects are produced follows that given by Crookes[2], to explain the visual sensations accompanying saccadic movements across a television screen.

The effects are best seen in a darkened room, and are particularly clear for downward movements which start a few centimetres above the line, and end on the line, in which case the overshoot is seen just above the level of the line. If much larger saccades, of about 30°, are made, starting above the oscilloscope and ending on the line, the overshoot is seen to be only slightly larger than for 3° saccades (average values ½° and ½° respectively). With a double-beam instru-

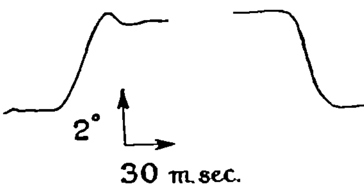

Fig. 1. Vertical saccades recorded with capacitance gauge

ment, two parallel curves appear to be traced out by the two spots.

When observation is made by reflexion in a mirror, so that the oscilloscope spot appears to travel from right to left, the traces are now reversed. Horizontal eye movements can be examined by either tilting the head, leaving the instrument fixed, or by rotating the oscilloscope so that the line is vertical.

Twelve subjects have perceived the effects, without difficulty, and have been able to estimate the duration of their own saccades and the dependence on the amplitude of the saccade. Reports differ slightly in the amount of overshoot, but agree that it is larger in downward movements, and that its magnitude is largely independent of the extent of the movement. In horizontal saccades this asymmetry appears to be less pronounced.

These subjective observations, in which there is no interference with the eye in any way, are consistent with my own experimental results, and with those of Byford[3], who has recorded eye movements by attaching a lamp to a contact lens. They do not support the model of saccadic eye movements put forward by Westheimer[4], on the basis of his own measurements.

A detailed analysis of the overshoot phenomenon is to be reported elsewhere.

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Breakdown of Seminal Glycerylphosphorylcholine by Secretions of the Female Reproductive Tract

IN the course of experiments on the metabolism of glycerol by ram spermatozoa, the seminal plasma was found to contain high concentrations of a substance estimating as glycerol[1]. This was identified as a glycerol complex, namely, glycerylphosphorylcholine, which was shown to occur in ram seminal plasma in a concentration of more than 1 per cent, and in the semen of the bull, goat, stallion, man and rabbit in lesser amounts[2]. Despite its high concentration in the seminal plasma, however, ram spermatozoa are unable to metabolize glycerylphosphorylcholine[2], and its physiological significance has hitherto been unknown.

On the other hand, it is well established that ram spermatozoa can utilize free glycerol, or phosphoglycerol, under aerobic conditions with the formation of lactic acid, which can be further oxidized to carbon dioxide and water[1,3]. It occurred to us, therefore, that there might be an enzyme in the secretions of the female tract capable of splitting choline from the glycerylphosphorylcholine molecule and thus liberating glycerol or phosphoglycerol that could be utilized by the spermatozoa, since conditions in the female tract are believed to be essentially aerobic[4]. This has proved to be so and the evidence is outlined in this preliminary communication.

Secretions were expressed from the uterus and Fallopian tubes of freshly slaughtered ewes, or washed out with Ringer solution. When 0·5 ml. of this fluid was incubated at 37° C., either overnight (7 experiments) or for 3 hr. (14 experiments), with 0·1–0·5 ml. of ram seminal plasma, a highly significant ($t = 5{\cdot}2$, d.f. $= 12$, $P < 0{\cdot}001$; and $t = 4{\cdot}5$, d.f. $= 26$, $P < 0{\cdot}001$ respectively), though somewhat variable, increase in free choline, as estimated by methods previously described[2], occurred. On the average, about 30 per cent of the glycerylphosphorylcholine was broken down in 3 hr. and about 50 per cent overnight. Thus appreciable quantities of metabolizable end-products (namely, glycerol or phosphoglycerol) are likely to be produced following the introduction of seminal glycerylphosphorylcholine into the female tract. The variability in free choline liberated is not unexpected, since it was only possible in these preliminary tests to calculate the results in terms of the volume of fluid washed from the female tract rather than on the basis of dry weight or protein content. Large variation in the composition of the uterine fluid itself might also be expected, depending on the stage of œstrous cycle[5].

No significant increase in free choline was found when the secretions of the reproductive tract of the ewe, or the seminal plasma of the ram, were incubated separately. The increase in free choline on mixing the secretions followed a fairly linear course during 3 hr., and could be almost exactly accounted for by the disappearance of glycerylphosphorylcholine. Thus, in three experiments a mean of 426 μgm. of free choline was liberated per ml. of incubation medium and 392 μgm. of glycerylphosphorylcholine–choline lost.

The liberation of free choline is clearly not due to bacteria, since it was unaffected by bacteriostatic concentrations of a mixture of penicillin and streptomycin. On the other hand, the increase in free choline was completely abolished by heating the secretions of the female tract for 5 min. at 100° C., suggesting that an enzyme, probably a diesterase similar to that found in the liver[6], is responsible. Similar investigations have shown that the enzyme is also present in the secretions of the reproductive tract of the cow and the sow.

In view of the apparent widespread occurrence of this enzyme in the female secretions, it is suggested that glycerylphosphorylcholine might be an important source of energy for spermatozoa in the female tract after copulation, despite the fact that spermatozoa themselves may not be capable of breaking down the intact molecule. It is interesting in this regard to compare glycerylphosphorylcholine with the better-known seminal constituent, fructose[7]. This sugar, which is readily utilized by spermatozoa, is produced by the seminal vesicles or prostate and is not normally mixed with the spermatozoa until after ejaculation[7]. Glycerylphosphorylcholine, on the other hand, is chiefly found in the epididymal fluid of the larger species[2], and, although always in intimate contact with the spermatozoa, can only be utilized by them after the semen has come into contact with the female secretions. The occurrence of a glycerylphosphorylcholine-splitting enzyme in the female secretions would seem to constitute a rather novel method of ensuring that spermatozoa have an additional source of energy available to them in the female tract.

The enzyme is now being further characterized, and full details of the work will be published elsewhere.

We are indebted to Prof. C. W. Emmens for his interest in this work, and to Mr. P. Bryden of

the New South Wales State abattoirs for his co-operation in obtaining experimental material. The work has been supported by grants from the Planned Parenthood Federation of America and the Wool Research Fund.

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Lipoprotein Composition of the Film lining the Lung

SMALL bubbles (~ 50μ diam.) in air-saturated water usually contract and disappear within a few minutes. In similar circumstances, however, small bubbles derived from animal lungs are stable. This is due[1] to their surface tension being reduced to 0·05 dyne/cm. or less by an insoluble surface film. This film is derived from the original film lining the lung alveoli; its (oven-dry) mass is about $6 \cdot 4 \times 10^{-7}$ gm./cm.[2]. *In situ* it annuls the tension of the sharply curved alveolar surface, which would otherwise give rise to transudation from the blood or to atelectasis[2]. For various reasons the film was at first thought to consist of mucoprotein.

Specimens of the substance lining the bubbles have now been isolated. Water was forced down the main bronchus into the excised lung of a cow, and was then allowed to well out, laden with bubbles. These bubbles were then washed with ten changes of water, so as to remove all dissolved matter and suspended solids. A sample of the washed froth, dried at 40° C., left a pale brown solid of waxy consistency.

This residue was examined in the form of a cast film on a potassium bromide plate by infra-red absorption spectroscopy, a model 137 Perkin–Elmer spectrophotometer being used. The spectrum, and that of a mixture of purified egg lecithin (95 per cent) and gelatin (5 per cent) for comparison, are shown in Fig. 1. Qualitatively, the curves are identical. The relative intensities of the ester carbonyl bands at 5·75μ and the amide bands at 6·1μ and 6·5μ in the two spectra suggest that the proportion of protein in the bubble lining is about 1 per cent. The presence of small amounts (< 1 per cent) of other lipids or of carbohydrates cannot be excluded, but it is clear that the lining film consists in the main of a lecithin–protein complex; the very low value of the protein content suggests that some of the lecithin present may be adventitious.

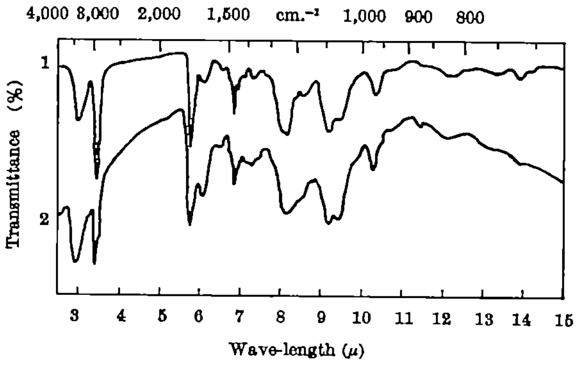

Fig. 1. Percentage transmission (on arbitrary scale) of infra-red radiation through (curve 1) lung bubble lining substance and (curve 2) mixture of 95 per cent lecithin and 5 per cent gelatin

Small stable bubbles have also been obtained from other sources. They are formed, for example, when dry powders of a bacterial lipopolysaccharide[3], of thromboplastic lipoprotein[4], or of acetone-washed egg yolk, are wetted. They may also be obtained by wetting acetone-dried or freeze-dried tissues (muscle, brain, etc.).

Lipoprotein complexes are known to form readily[5,6]. When, for example, lecithin (4 gm.) and gelatin (1 gm.) are ground to a paste with water, and the paste is dried with acetone, a leathery porous substance results; this, when wetted and squeezed under water, yields stable bubbles. It may therefore be that some of the bubble-stabilizing substances found in dried tissues are artefacts which are not present *in vivo*.

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Effect of Exposure to Sun and Exercise on Heat Tolerance Coefficient in Murrah Buffalo Calves

IN continuation of our previous work[1], this experiment was designed to find out the effect of exposure to sun and exercise on heat tolerance coefficient. Eight Murrah buffalo calves, varying between 8 and 12 months of age, constituted the experimental material. These calves were randomly divided into two groups of four each. One group was exposed to sun, while the other ran at a speed of four miles per hour as recorded by the distance covered in a given time. The calves in each group acted as their own control. These experiments were conducted at 12 noon for half an hour on six consecutive hottest days in the month of May. The atmospheric temperature during test hours ranged between 92 and 105° F. and relative humidity between 28 and 47 per cent. The conditions of management of these calves has been given in detail[2].

The heat-tolerance coefficient was calculated daily for each animal, as control and after exposing them to sun or exercise, by Rhoad's formula[3]. The variations were analysed statistically[4].

Table 1 indicates mean value, variance, standard deviation, and coefficient of variation of the atmospheric temperature, relative humidity and heat-tolerance coefficient. There seems to be a degree of consistency in the mean values of the controls of the

Table 1. Heat Tolerance Coefficient

	Temperature		Humidity		First group		Second group	
	Under shade	In sun	Under shade	In sun	Control (in shade)	Exposed to sun	Control (in shade)	Put to exercise
Mean	93 66	98 00	42·00	32 66	92·38	80·80	94·04	65·00
Variance	1·48	16·40	10·40	43·48	62·14	68·86	19 52	185·78
Standard deviation	1·21	4·04	3 22	6 52	7·88	9·31	4·42	13·62
Coefficient of variance (per cent)	1·29	4 12	7·66	20·11	8·53	11·5	4 90	20 95

two groups, but F value indicates ($F_{24,24} = 3 \cdot 1$; $P_{1\%} = 2 \cdot 66$) that the variation between the controls of the two groups is significant. As such these values could not be pooled together to test the differences between the two different groups. However, the variance ratio in the first and the second group when compared with their own controls were 1·39 and 9·51 respectively ($F_{22,22}$ 5 per cent $= 1 \cdot 98$). Since variance of heat tolerance coefficient in the first group was not significant, the t test was applied for testing the differences between the two means ($t_{24} = 2 \cdot 39$; P_{24} 5 per cent $= 2 \cdot 06$). These two mean values were found to be significant.

Table 2 indicates that there were significant differences in heat tolerance coefficient of the calves before and after exposure to the sun. The calves of the other group when put to exercise showed significant differences in their heat tolerance between themselves, between days and between treatment. Every animal behaved differently for its capacity to withstand the thermal stress on different days to the exercise given.

Table 2. Analysis of Variance

Sources of variation	*d.f.*	Mean square	
		First group Effect of exposure to sun	Second group Effect of exercise
Between animals	8	158 58	61·40*
Between days	5	187 08	375·38*
Between treatment	1	1,598 52*	10,120·84*
Between animal × days	15	43·62	29·21*
Between animal × treatment	3	47·63	31·83*
Between days × treatment	5	13·62	403 45*
Between animal × days × treatment	15	76 65	7 23

* Significant at 1 per cent level.

The buffalo calves after exercise were observed seeking the shade and in distress characterized by licking, panting and general restlessness. Drooling appeared when the increased respiratory activity proved unsuccessful in stabilizing body temperature. These are physical homœothermic mechanisms which ease the burden of heat load on the animal[5]. The ability of the buffalo calves to withstand the tropical heat, especially when exposed to the sun or put to exercise, seemed to decrease, perhaps because of the increased heat production and absorption[6]. The black pigmented hide absorbs the infra-red rays of solar radiation and thus limits the animal's adaptability to exposure to sunlight. In the shade, however, the black coloration aids heat dissipation[7]. Adaptation to hot humid climate is characteristic of the buffaloes. The thick skin of the buffalo calves may prevent heating of the body from hot air and by radiation from the sun or hot ground. The buffalo calves, although surviving in hot zones when humidity is high, essentially require shade and cooling systems for their comfort[8].

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HÆMATOLOGY

A Notation for the Gm Groups

The following met in Rouen in April 1960 to discuss notation and other matters regarding the Gm serum protein groups of man: A. Eyquem, Institut Pasteur, Paris; H. Fudenberg, Rockefeller Institute, New York; R. Grubb, Bacteriological Institute, Lund; M. Harboe, Institute for Thrombosis Research, Oslo; S. Lawler, Galton Laboratory, London; J. Moullec, Centre National de Transfusion Sanguine, Paris, represented by C. Henry; L. Podliachouk, Institute Pasteur, Paris; C. Ropartz, Centre Départemental de Transfusion Sanguine, Rouen; A. Steinberg, Western Reserve University, Cleveland; A. Tiilikainen, Department of Serology and Bacteriology, University of Helsinki. The following statement has been prepared.

It is increasingly apparent that the Gm system is complex and our aim is to minimize confusion originating in differing nomenclature or standards.

To be accepted as part of the Gm system a character must be shown to be contained in the gamma-globulin fraction, and its relationship to the known Gm factors demonstrated by genetical and/or population studies.

The characters to be considered at the present state of knowledge are Gm(a), Gm(b), Gm(x) and Gm-like as originally described in references 1–6 respectively.

Symbols for genotypes and phenotypes should be clearly distinguishable. In conformity with the suggestions of Ford[7], we will adhere for Gm notation to the two following rules: (1) the use of italics for gene symbols and the use of ordinary type for phenotypes; (2) the use of suffixes for genes, but not for phenotypes. Example: Gm^a for the gene, Gm(a +) for the phenotype.

The use of gene symbols containing the suffixes a_1 and a_2 for specified characters has been suggested[8]. This proposal is now withdrawn. At the present state of knowledge no further suggestions for the gene symbols seem warranted.

The notation which will be used for the phenotypes now known is as follows: Gm(a + b + x +), Gm(a + b + x −), Gm(a + b − x +), Gm(a + b − x −), Gm(a − b + x +) (Henningsen, unpublished observations confirmed by Harboe), Gm(a − b + x −), Gm(a − b − x −). The type Gm(a − b − x +) has not yet been encountered. This notation can readily be expanded to adopt new phenotypes as they may be found and/or be included in the Gm system. To denote the Gm-like character, Gm-like (+) or Gm-like (−) is added; for example, Gm(a + b + x −) Gm-like (+). The term Gm-like is regarded as temporary. So-called intermediates may be signified by ± ; for example, Gm(a ±). The gamma-globulin-levels in sera of unusual phenotypes should be determined and given.

The terms anti-Gm(a), anti-Gm(b) and anti-Gm(x), to which the classical noncommittal term 'reagin' may be added, will be used to denote the specificities of the substances (usually, if not always, macroglobulins) causing the agglutination of red cells coated with human incomplete antibodies.

Samples of normal human sera of at least some of the known Gm-types, which can be used as standards, will be supplied on request by me. Standard Gm-like (+) and Gm-like (−) samples are obtainable from A. Steinberg, Department of Human Genetics, Western Reserve University, Cleveland 6, Ohio, U.S.

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Formation of Hæmosiderin and its Relation to Ferritin

Support for the hypothesis that the protein moiety of hæmosiderin is the same as that of ferritin can be found in the literature[1,2]. Iron tetrad (or, more recently, 'hexad'[3,4]) structures in a protein matrix characteristic of ferritin have been reported in electron photomicrographs of tissue sections variously interpreted as ferritin, hæmosiderin, aggregates of ferritin, etc.[3,5,6]. It is not uncommon, therefore, to consider both ferritin and hæmosiderin as being more or less identical. However, the results of our biochemical and histochemical work lead to opposite conclusions[7,8].

Before commenting further, it should be emphasized that in biochemical terms, as originally defined by Laufberger[9], the fundamental property that distinguishes ferritin from hæmosiderin is the solubility of ferritin in water. It must also be recognized, despite the common misconception to the contrary, that ferritin does take the Prussian blue stain[10]. Failure to recognize and to apply these principles introduces the possibility that 'hæmosiderin' in tissue sections might consist partially or entirely of ferritin.

We have observed with biochemical and histochemical techniques that, as the water-extractable iron (ferritin) of rabbit liver approaches 200 mgm. per 100 gm. of tissue, Prussian blue-positive granules (hæmosiderin) form in the parenchymal cells. This phenomenon is clearly observed when the iron is administered as iron dextran; with saccharated iron oxide, the phenomenon is also reproducible relative to the parenchymal cells. In the Kupffer cells, there is in addition, with the latter preparation an accumulation of stainable insoluble iron at an early stage. This Kupffer cell iron differs in several respects from that of the parenchymal cells; it is not under consideration at present.

At levels of iron load just preceding that at which the typical Prussian blue-positive hæmosiderin granules appear, the formation of several intracytoplasmic vacuoles in the parenchymatous cells adjacent to the portal regions can be observed. These vacuoles are 1–2μ in diameter, have irregular borders, tend to be in proximity to the nuclei and, most important, give a faint blue homogeneous colour on staining for iron. As the iron load is increased, the larger of these vacuoles begin to show typical dense, dark-staining Prussian blue granules. At this point, one can still identify faint light blue-staining material at the periphery of these vacuoles. With increasing iron load, only typical Prussian blue-positive hæmosiderin granules are evident. The light blue material observed at earlier stages can be removed by water extraction of cubes of liver (1–2 $mm.^3$). The quantity of iron recovered in the extract corresponds closely to the ferritin content of the liver, as determined by biochemical methods. The dense, dark blue-staining granules remain unaltered. We have recorded these events by colour photomicrography; this work will be published elsewhere.

From the above, the lack of clear-cut distinction between hæmosiderin and ferritin by electron and/or light microscopy on tissues which have not had the ferritin removed by water extraction can be explained. For example, a recent electron photomicrograph by Bessis and Breton-Gorius[11] shows sections composed of electron-dense particles in a matrix, linearly arranged in a crystal lattice. This, as well as publications by Richter[12] and Kerr and Muir[3], also show aggregates of electron-dense iron particles with little or no matrix. They are variously interpreted as being ferritin crystals, different forms of hæmosiderin, aggregated ferritin, etc. As our work on granules isolated after extraction of ferritin indicated that the two compounds were chemically distinct, we submitted our preparations to Dr. G. W. Richter for electron microscopy. The electronmicrographs showed closely aggregated electron-dense particles, but neither ferritin crystals nor even isolated 'tetrads' were evident. This collaborative work is being extended and will be published separately.

These observations indicate that hæmosiderin and ferritin are not only chemically but are also structurally dissimilar. They also suggest strongly that the hæmosiderin granule, at least of the rabbit liver parenchymal cell: (1) is a relatively amorphous condensation of electron-dense iron particles into an essentially protein-free aggregate; (2) that it consists mainly of ferric hydroxide; (3) that it originates in liver cell vacuoles through a process of degradation of the protein matrix of ferritin; (4) that the linearly arranged iron particles in a matrix correspond to the faint blue-staining material we have observed histochemically in parenchymal cell vacuoles. Because of the water-solubility of the latter material, we conclude it represents ferritin. This concept leaves room for intermediate stages between native ferritin and the apoferritin-free aggregates of ferric hydroxide particles.

Therefore it is recommended, in order to bring anatomical and biochemical nomenclature into agreement, that the term 'hæmosiderin' should be applied only when the compound under investigation is known to be water-insoluble. This division may be arbitrary in an anatomical sense, but it is easily and accurately applied and will preclude the misconception that hæmosiderin and ferritin are identical.

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An Attempt to produce Hæmolytic Disease in Lambs

RENDEL[1] attempted to produce hæmolytic disease of the new-born lamb by injecting 8 pregnant ewes with blood taken from the rams to which they were mated. As a result, 4 of the ewes produced immune antibodies which reacted with the red cells of their mates; but their lambs did not develop hæmolytic disease even though at least 2 of them ingested colostrum-containing antibodies which reacted weakly *in vitro* with their respective red cells. In these cases, no antibody could be detected on the lambs' red cells, and Rendel concluded that if hæmolytic disease occurred in lambs it must be rare.

This communication describes an experiment carried out on similar lines to those of Rendel. A ewe was chosen which one year previously had been immunized with the blood of a certain ram and which had made a strong immune antibody to the ram's red cells. This ewe was mated to the ram, and during the later stages of pregnancy was given a booster injection of the ram's blood which resulted in a sharp rise in serum antibody titre from only a trace reaction to a titre of 1/16,000. Twin female lambs were born to the ewe after the normal pregnancy time, and blood samples were taken from them before they had sucked, and at 4, 7, 10 and 24 hr. after sucking and at intervals thereafter. In addition, blood serum and milk samples were taken from the ewe before and after parturition.

Both the serum and colostrum samples taken from the ewe at the time of parturition were titrated against the lambs' precolostrum red cells, using a simple hæmolytic test. To 1 drop of a 2 per cent red cell suspension in saline, 1 drop of the serum or colostrum at the appropriate dilution was added. These were mixed and left for 15 min. when 1 drop of undiluted rabbit complement (rabbit serum, absorbed with sheep red cells) was added. The test was left at 32° C., shaken at hourly intervals and read after 4 hr. The highest dilution of antiserum showing definite hæmolysis was taken as a measure of the strength of the antibody. Using this test, it was found that the dam's serum and colostrum reacted with the red cells of both lambs to a titre of about 1/8,192–1/16,384. The samples of red cells taken from the lambs were therefore tested to see whether they were 'sensitized'. They were washed once in saline and to 1 drop of a 2 per cent saline suspension of the red cells, 2 drops of rabbit complement diluted ½ were added and the test incubated at 32° C. Readings were taken after 1 hr. No hæmolysis was observed in the precolostrum or 4-hr. red cell samples; but the red cells taken from the lambs 7 and 10 hr. after sucking showed partial hæmolysis. The 24-hr. samples showed complete hæmolysis as did samples taken 5 days after birth. Nine days after birth the lambs' red cells again showed partial hæmolysis on the addition of complement and by the seventeenth day there was no evidence of sensitization. The hæmatocrit of the blood samples fell sharply during the first 5 days after birth (from 44 to 27 per cent in one lamb and from 47 to 25 per cent in the other lamb); but a similar drop was observed in blood samples taken from control lambs during the first week after birth, so this was considered to be of no significance. An osmotic fragility[2] test was made on the red cells of one of the lambs taken 5 days after birth, that is, at a time when the cells were well coated with antibody, but there was no evidence that their fragility was in any way different

Table 1. ANTIBODY TITRES AGAINST LAMBS' RED CELLS

Time of sample	Lamb No. *M*292			Lamb No. *M*293		
	Serum titre* of dam	Colostrum titre* of dam	Serum titre* of lamb No. *M*292	Serum titre* of dam	Colostrum titre* of dam	Serum titre* of lamb No. *M*293
Pre-sucking	8,192	16,384	0	16,384	16,384	0
4 hr. post-sucking	—	—	0	—	—	2
7 hr. ,,	—	8,192	0	—	8,192	16
10 hr. ,,	—	—	64	—	—	64
24 hr. ,,	—	1,024	64	—	1,024	64
2 days ,,	8,192	256	32	8,192	128	64
5 days ,,	—	—	8	—	—	8
9 days ,,	2,048	—	0	2,048	—	0
17 days ,,	—	—	0	—	—	0
23 days ,,	1,024	—	0	1,024	—	0

—, Not tested. * Titre expressed as the reciprocal of the highest dilution of antiserum giving definite hæmolysis.

from that of the red cells of the control lambs of comparable age. Both lambs in fact showed no abnormal symptoms and grew and developed normally.

When the lambs were about 2 months old, their red cells were tested against titrations of their own sera, and against their dam's serum, and colostrum samples taken during the first days after birth. Table 1 shows the results. Antibody appeared in both lambs' sera at about the same time that their red cells began to show sensitization, reached maximum levels at 10 hr. and then declined in titre until by the ninth day none was detectable.

0·4 ml. of red cells from one of the 2-month-old lambs was labelled with chromium-51 (ref. 3), incubated for 20 min. at 37° C. in 8 ml. of a 1-in-4 dilution of the dam's serum taken at parturition, washed and injected back into the same lamb. A blood sample taken 5 min. after the injection showed only a trace of radioactivity, indicating that the injected red cells had all been quickly eliminated from the circulation. This experiment was repeated, only this time the lamb's red cells were incubated in a 1-in-4 dilution of its own serum taken 1 day after birth when it had an antibody titre of 1/64. The red cells were again quickly eliminated from the circulation.

The situation was therefore found where two lambs failed to show any symptoms of hæmolytic disease even though their red cells were strongly sensitized by antibodies ingested from the dam via the colostrum. A similar condition was reported in calves by Chavez[4], who fed a calf with colostrum to which had been added anti-ox antibody produced in rabbits, and demonstrated that its red cells became coated with antibody. The calf showed no symptoms of hæmolytic disease. Kiddy, Stone, Tyler and Casida[5] isoimmunized cows with a bull's blood; but although the dams' sera and colostra reacted with the calves' red cells in their experiments, no antibody to the calves' own red cells could be found in the calves' sera and no antibody could be detected on their red cells. From these findings they suggested that the mechanism protecting the new-born calf was the absorption or neutralization of ingested antibodies by tissue or soluble antigens, thus preventing the antibodies from reaching the calf's erythrocytes and causing their destruction. This could not be the explanation of the failure to produce hæmolytic disease in the lambs, for not only were the red cells coated with antibody, but at the time at which there was maximum *in vivo* sensitization there was also free antibody circulating in the plasma. The fact that the lamb's red cells when sensitized with this free antibody were rapidly eliminated from the circulation of the lamb when it was 2 months old would suggest that the protective mechanism might lie in the mode of action of the reticulo-endothelial system of the newly born lamb or a deficiency in some serum factor which causes destruction of sensitized cells.

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RADIOBIOLOGY

Strontium-90 in Human Teeth

THE ultimate concern in strontium-90 fall-out monitoring is the amount of the nuclide assimilated in man. Several world-wide studies have been made to determine the concentration of this fission-product in human bone. Kulp[1] defined the strontium-90 content of bones of children and adults up to 1959, while Bryant published similar United Kingdom bone results for 1958 [2,3] and early 1959 [4].

Since bone is usually obtained at autopsy, sample collection is limited and complicated for local studies. Strontium-90 analysis of human teeth, especially deciduous teeth, is simpler from the point of view of collection and preparation.

Kalchar[5] first suggested a world-wide deciduous tooth radiation census. He stated that strontium-90 assays of teeth would reflect the fall-out level at or near the time of birth. There is some evidence that teeth may also reflect, to a lesser degree, fall-out levels up to the time of removal. Armstrong[6] and other investigators found calcium-45 and phosphorus-32 in mature animal teeth shortly after administration of the radionuclides. The relative amounts of uptake of these radionuclides were: bone > dentine > enamel.

The growth chronology of deciduous and permanent teeth was reported by Wheeler[7]. Each type of tooth has a different chronology, and there are also variations between individuals. Deciduous teeth begin to form before birth, but enamel and roots are not completed for many months. Permanent teeth are not completely calcified for several years after eruption.

Only a few teeth analyses have been reported, due to insensitive methods of analysis and relatively small amounts of strontium-90 in teeth formed in the early 1950's. The highest result of three analyses reported by Hardy[8] was 0·79 ± 0·12 μμc. strontium-90/gm. calcium.

Bryant[9] recently reported the strontium-90 content of sound 9- to 13-year permanent premolars and 16- to 21-year third molars. The strontium-90 concentration for both groups lay in the range 0·1–0·4 μμc./gm. Although there was a rough inverse correlation with age, he reported that the material was too limited to be biologically significant.

The method used by Bryant is typical of most strontium-90 procedures used to date. After addition of strontium carrier, the strontium-90 and natural calcium from 40-gm. batches of teeth were precipitated as oxalates and then as carbonates. Strontium was separated from calcium by repeated precipitations as nitrate. Strontium carbonate was finally precipitated and mounted for counting.

Teeth results reported here were obtained by an improved method of analysis. One hundred and twenty-one teeth were collected in late 1959 and combined to 81 samples before analysis. After a minimum of analytical steps, the final strontium-90 content was determined by counting carrier-free yttrium-90. The method was thoroughly investigated and reported in a previous general fall-out survey[10]. Preparation of teeth included an initial 3-min. soaking in 1 : 10 hydrochloric acid to remove surface film, followed by rinsing with distilled water. Fillings were removed and teeth were air-dried overnight before weighing.

Initial experiments proved that direct dissolution of teeth and co-precipitation of calcium and strontium as oxalates were preferable to ashing. Teeth were dissolved in 50 ml. of 1 : 1 hydrochloric acid. After complete solution, the sample volume was increased to 600 ml. by addition of distilled water. Three grams of oxalic acid were added, and the solution was heated to 80–90° C. Oxalates were precipitated by gradual addition of 1 : 1 ammonium hydroxide, while stirring, to *p*H 5–6. After overnight digestion, the precipitate was separated by vacuum filtration through Whatman No. 42 filter paper. The oxalates were fired to oxides in a muffle furnace at 650° C. The final solid was then dissolved in 50 ml. of 0·08 *N* hydrochloric acid, prior to strontium-90 analysis.

Strontium-90 analysis was obtained by counting the carrier-free yttrium-90 daughter, after an extraction procedure. The 50-ml. sample was first stripped of yttrium-90 by two extractions with equal volumes of 20 per cent di-2-ethylhexyl phosphoric acid in toluene. The yttrium-90 daughter of strontium-90 was allowed to build up to the equilibrium-level over a period of two weeks and was then extracted from the original sample with 5 per cent di-2-ethylhexyl phosphoric acid. Yttrium-90 was removed from the organic layer by extraction with 3 *N* nitric acid. The acid was then evaporated and plancheted. All samples were counted two or more times in a low-background counter to verify the yttrium-90 decay.

Results of this preliminary analysis are shown in Table 1. Due to the small number of samples in each age-group, the types of teeth were not reported. The large errors shown in the last column are due to analyses of individual teeth weighing as little as 0·1 gm. This experience indicates that further sample composites should weigh not less than 2 gm.

Table 1. STRONTIUM-90 ANALYSES OF 121 HUMAN TEETH

Age of donors	No. of teeth	No. of assays	d./m. ^{90}Sr/gm. tooth		
			Maximum	Minimum*	Average†
4	5	4	4·4	1·0	2 6 ± 0 6
6	6	8	4·7	0·2	2 1 ± 2 1
7	12	8	6 2	neg.	1·6 ± 0 8
8	17	7	8 2	0·1	1 5 ± 0·8
9 and 10	19	12	6 0	neg.	0·8 ± 0·4
11 and 12	11	9	2 8	neg.	0 8 ± 0 8
13 and 14	7	5	3 1	neg.	0 8 ± 0 2
15–18	5	5	1 4	0·2	0·8 ± 0·3
19 and 20	8	7	0 8	0 1	0·5 ± 0·3
21–30	17	11	1·8	neg.	0 5 ± 0·3
31 and up	14	10	1·3	neg.	0·4 ± 0·2

* Negative assays are those that counted equal to or less than reagent blanks.

† The standard 120-min. counting error is reported for the average assay.

d./m., Disintegrations/min.

A statistical analysis indicates that there is a definite inverse correlation between strontium-90 concentration and age. The significance test for linear correlation ($r = -0{\cdot}32$), based on 81 independent paired observations, reveals that the probability is less than 0·01 that this inverse relationship does not exist. The trend is expressed better, however, in an exponential form of the type :

$$S_c = B \exp(-kA)$$

where : S_c = strontium-90 concentration ; A = age ; B and k = constants.

The strontium-90 results for teeth are compared with those of bone in Fig. 1. The disintegrations/min. values were corrected to μμc./gm. calcium by assuming 40 per cent calcium in teeth. The trend is similar to the probable distribution of strontium-90 in Western man as reported by Kulp[1], and also to the results obtained by Bryant for bone collected in 1958 [2,3].

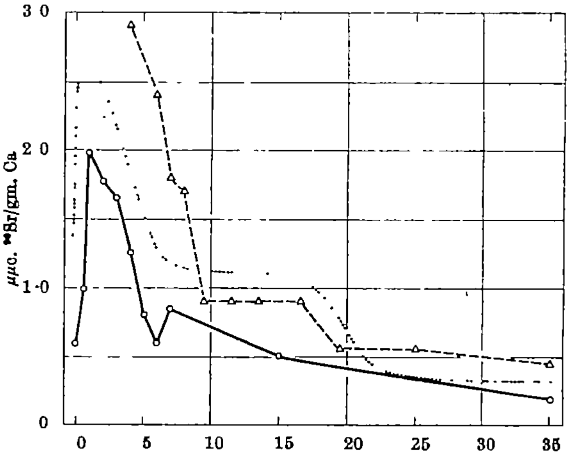

Fig. 1. Strontium-90 in teeth and bones. —△—, Strontium-90 in teeth, 1959 assays; . . . , strontium-90 in bones, 1959, probable (Kulp); —○—, strontium-90 in bones,1958 assays (Bryant)

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A New Effect of Ionizing Radiation on Nucleoprotein

An aqueous solution of nucleoprotein, concentration 12 mgm./ml., prepared from calf thymus by the method of Kay, Simmons and Dounce[1], was irradiated with high-energy electrons to a dose of 4 × 10^6 rads. The dose was given in 4 min., and was assumed to be delivered to an oxygen-free solution, the initial 2 per cent of the dose using up all dissolved oxygen[2]. It was noted that immediately following irradiation a white suspension had been formed, and this afterwards collected at the bottom of the tube on standing. When the irradiated and control solutions were analysed it was found that in addition to producing a precipitate, irradiation had degraded amino-acids, purine and pyrimidine bases and deoxyribose sugar. Details of these degradations will be published elsewhere; the present report is concerned with the nature of the precipitate.

No precipitate had been observed when calf thymus deoxyribonucleic acid (DNA) was irradiated under similar conditions[3]. The precipitate was not 'denatured protein' since no precipitate was formed when an aqueous solution of calf thymus histone was irradiated under similar conditions. It was possible that the precipitate could be 'cross-linked' material derived from both the protein and nucleic acid moieties. This possibility would be checked most suitably by irradiating a mixture of histone and DNA solutions. Unfortunately, mixing solutions of DNA (5 mgm./ml.) and histone (7 mgm./ml.) leads promptly to precipitation, presumably of a nucleoprotein complex[4]. As a substitute experiment, a solution containing histone and a mixture of deoxyribonucleotides was prepared and irradiated as described above. No precipitate was observed after irradiation, suggesting that cross-linking did not produce the precipitate obtained on irradiating nucleoprotein.

The most notable property of the precipitate obtained from irradiated nucleoprotein was its insolubility in water, N hydrochloric acid, N sodium hydroxide and $2M$ sodium chloride. The possible nature of the precipitate was discussed with Dr. K. S. Kirby, who suggested, in the light of his work with P. M. Frearson[5], that the precipitate was an acidic protein, and further that it may have small amounts of nucleotides attached to it. The precipitate was analysed as described here, and Kirby's predictions were confirmed. The precipitate contained an acidic protein with purine and pyrimidine bases attached, and so it is not derived from the bulk of the protein present (histone) in the nucleoprotein, but from a residual protein of the type originally described by Mirsky and Ris[6]. The production of this precipitate by ionizing radiation confirms the presence of an acidic protein in the structure of nucleoprotein. Further, in view of the possible role of the acidic protein in maintaining the structure of nucleoprotein[6], and as a site for enzymatic action[7], precipitation of the protein would affect profoundly the structure and function of the nucleoprotein in an irradiated cell.

Solutions of nucleoprotein, after irradiation, were spun, in a bench centrifuge, in the cold, and the clear supernatant solution decanted off. The precipitate was shaken in distilled water, and re-centrifuged. After washing five times the precipitate was freeze-dried. A weighed sample of the dried precipitate was hydrolysed in perchloric acid (72 per cent) at 100° C. for 1 hr. and analysed for phosphorus[8] and for purine and pyrimidine bases[9]. Large amounts of phosphorus were found (Table 1), and assuming it is present as phosphate, accounts for 70 per cent, by weight, of the precipitate. Small amounts of adenine, guanine, cytosine and thymine were found in the hydrolysate (Table 1). The base ratios were different from those of calf thymus DNA, amounts of guanine and cytosine being relatively greater (double) in the precipitate.

A second sample of the precipitate was hydrolysed in $6N$ hydrochloric acid, at 110° C. for 24 hr. and the hydrolysate analysed for amino-acids by two-dimensional paper chromatography. When the chromatograms were dipped into a solution of ninhydrin a large number of ninhydrin-positive spots appeared, their pattern being identical with that obtained when a sample of unirradiated nucleoprotein was analysed in the same way. The ninhydrin-positive spots were eluted and the amounts of amino-acids determined spectrophotometrically[10]. The relative amounts of amino-acids were similar to those for an unirradiated solution of calf thymus nucleoprotein except that the amounts of glutamic and aspartic acids were approximately 50 per cent greater in the precipitate than in the control. The total amount of amino-acids was approximately that given in Table 1, this value being the remainder when the weight of the purines and pyrimidines, as nucleotides, and of phosphorus, as phosphate, was subtracted from the weight of the precipitate.

Table 1. ANALYSIS OF PRECIPITATE FROM IRRADIATED NUCLEOPROTEIN

		μM/mgm.
Bases:	Adenine	0 05
	Guanine	0·08
	Cytosine	0·07
	Thymine	0·08
	Total	0·28
Phosphorus		6·74
Amino-acids (molecular weight = 127)		2·2

The results obtained for the precipitate are consistent with Kirby's postulate[11] of an acidic protein attached to the nucleic acid moiety. The source of the large amounts of insoluble phosphate in the precipitate is not clear at present. It is Kirby and Frearson's experience that the acidic protein is insoluble and will remain in solution only when combined with DNA. If ionizing radiation severs the link between the acidic protein and the DNA, or breaks the DNA chain within a few nucleotides of the acidic protein, precipitation will promptly follow. There are two ways in which this can occur. Radiation can break the DNA chain, randomly along its length, and the acidic protein will be precipitated when breaks occur sufficiently close to it. In this case the G-value (molecules per 100 eV.) for formation of precipitate will be lower than the G-value for chain breaks by a factor which depends on how close to the protein a break needs to be for precipitation to occur. Alternatively, if energy introduced into DNA by ionization or excitation can migrate along DNA, as discussed recently by Prof. J. S. Mitchell[12], the migration will stop at the discontinuities which the acidic protein must represent in the regular structure of DNA. If the transferred energy is sufficient to break the nucleic acid chain, the acidic protein will be precipitated. If energy is readily transferred along the DNA, acidic protein will be precipitated by low doses of radiation.

Dounce[13] has recently discussed data which show that a residual protein is firmly bound to DNA, as first demonstrated by Mirsky and Ris[6]. While the nature of the binding has not been established, Kirby[14] and Dounce[13] have shown that the bonds are not ionic. Transition-group metals may be involved in a chelation-type bond between the acidic protein and the DNA (ref. 14), and the relatively large amounts of guanine are consistent with a bond of the type suggested by Kirby[14].

The amount of dried precipitate obtained was 0·5 mgm./ml. As 20 per cent of this is protein (see Table 1) the precipitate contains approximately 1·4 per cent of the total protein originally present. Before a G-value for formation of the precipitate can be calculated, it is necessary to determine how the yield of precipitate varies with dose, and also to obtain some estimate for the molecular weight of the acidic protein. Whatever the G-value may be, it is likely that this precipitation will play an important part in the biological effects of ionizing radiation, since it would alter so profoundly the structure and function of the nucleoprotein. Kaufman *et al.*[15] have studied several properties of nucleoprotein of onion roots, *Drosophila melanogaster* and calf thymus, before and after irradiation, and demonstrated degradation of the nucleoprotein with a few hundred r. of X-rays. More recently Hagen[16] has reported that whole-body irradiation of rats labilizes the thymus nucleoprotein. The precipitation described above is a possible mechanism for this labilization. Since Hagen obtained labilization with doses as low as 50 r., precipitation of the acidic protein would be an extremely radiosensitive reaction.

I am indebted to Dr. K. S. Kirby for his help in determining the nature of the precipitate. This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service. Financial support was also granted by the William Shepherd Fund.

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'Nitric Oxide—and Oxygen Effect' in X-irradiated *Shigella flexneri*

IN a number of cases, for example, bacteria and ascites tumour cells, it is known that nitric oxide increases radiation damage in anoxic conditions to a level comparable with that found in the presence of oxygen. The so-called oxygen effect is thus simulated by nitric oxide.

The starting point of the present investigations was the fact that the chemically very active gas nitric oxide has some effect on cell constituents, and the belief that this possibly, indeed probably, might become manifest after removal of free gas and subsequent X-irradiation. It appeared from the literature that the effect of an exposure of aqueous suspensions of bacteria to nitric oxide, followed by its removal prior to irradiation in oxygen-free nitrogen, had not been tested. While the present work was in progress, Howard-Flanders and Jockey[1] published results showing that this experiment had been carried out independently by them. However, their results obtained with the bubbling technique differ from the present ones obtained in a closed system in various respects not only in regard to the pre-treatment effect but also in some other points.

The results of the present series of experiments may be very briefly summarized as follows:

(*a*) Radiation sensitivity in the presence of nitric oxide is increased over that in nitrogen by a maximal factor of 3·2. A similar enhancement is found in oxygen and these results are in agreement with those of Howard-Flanders and Jockey.

(*b*) If the cells are pre-treated in nitric oxide for 30 min. at room temperature and irradiated in nitrogen after removal of free nitric oxide, sensitivity is again greater than in cells not pre-treated.

(*c*) Radiation sensitivity varies with the concentration of nitric oxide applied (up to a maximum value at 10 per cent nitric oxide in nitrogen), during or prior to irradiation. The minimal dose for 10 per cent survival is about 15 k.rads in the presence of the gas compared with about 20 k.rads after pre-treatment. For nitrogen controls this figure is 49 k.rads.

(*d*) Increasing duration of pre-treatment increases the subsequent radiation sensitivity.

(*e*) Cells kept in nitrogen (maximum period about 2 hr.) after pre-treatment with nitric oxide show a constant sensitivity.

(*f*) The pre-treatment effect is not due to residual free nitric oxide because of the evidence quoted in paragraphs (*c*) and (*d*) and also because of the high degree of consistency obtained for the radiation sensitivity in nitrogen.

The conclusion to be drawn from the present results is that the 'nitric oxide' effect seems largely caused by some latent, irreversible injury to unknown constituents of the cells with which nitric oxide may have combined. This injury is revealed on subsequent irradiation. The nitric oxide effect is thus different from the 'oxygen effect', which is only seen when irradiation occurs in the presence of the gas. Furthermore, the effect of time of contact with, and of the concentration of nitric oxide during, pre-treatment does not support the equivalence of nitric oxide and oxygen in their role in enhancing injury by radiation.

A detailed account of this investigation will be published in due course.

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Adaptation of a Chromatographic Technique for Autoradiography

A QUALITATIVE chromatographic technique has been previously described[1] which enables the investigation of simple compounds and their distribution in plant tissues to be accomplished without the immediate necessity of preparing extracts (for example, ether–water or alcohol). This method can be further extended by its use with radioactive isotopes and subsequent autoradiography.

Five day-old seedlings of *Phaseolus vulgaris* L., variety Improved Canadian Wonder (with radicles approximately 4–5 cm. long) were injected with 10 µl. of a solution containing 0·4 mc./µl. of sucrose uniformally labelled with carbon-14. In one batch of seedlings (*a*) the sucrose was injected with a micro-syringe into the thickest part of the radicle, and in another batch (*b*) it was injected into the distal end (with respect to the embryo) of one of the cotyledons. The injected seedlings were then allowed to continue to grow normally, each batch being sampled at 1 hr., 2 hr., 4 hr., 1 day, 2 days and 4 days after injection. The sample seedlings were subjected to the chromatographic technique on Whatman No. 3 chromatography paper. After development, using conventional methods and solvents, the chromatograms were dried and placed with sheets of Ilford *G* X-ray film in cassettes and allowed to remain in contact with the films for 3–4 weeks. After this time, radioactive compounds were detected by developing the X-ray plate and could be compared with the compounds on the chromatograms after these had been treated with specific reagents (in this case, ninhydrin to detect amino-acids followed by benzidine–trichloracetic acid to detect the saccharides).

The results showed that in the case of the embryo-injected seedlings (*a*), the sucrose pool in the embryo became labelled and also the pool of reducing sugars, glucose and fructose. Various amino-acids were rapidly labelled and also (high molecular weight) material which did not move from the starting line of the chromatogram. Eventually, after four days, only small amounts of the labelled saccharides and amino-acids remained although considerable quantities of these compounds (unlabelled) were still evident on the chromatograms (that is, in the embryo). The majority of the radioactivity was found to be associated with the material at the starting line of the chromatogram. In the case of the cotyledon-injected seedlings (*b*), the cotyledon sucrose pool was labelled, but the evidence suggested that only amino-acids and 'high-molecular weight' material became further labelled; no reducing sugars became labelled. However, there is little reducing sugar in the cotyledons during germination (approx. 0·05–0·1 per cent dry wt.)[2].

In both cases (*a*) and (*b*), the labelled sucrose was found to appear in the other parts of the plant as well as in the injected part, but to a lesser extent in case (*a*) than case (*b*). This observation could possibly mean that sucrose can be translocated in both directions in the plant (that is, cotyledons to embryo and vice versa); this is consistent with the findings of Rabideau and Burr[3], of Chen[4], and of Palmquist[5] who observed that solutes can be translocated in both directions in the stems of *P. vulgaris* and other plants. Nevertheless, the net flow of sucrose was in the direction cotyledons to embryo as would be expected.

In case (*b*), injected cotyledons, as well as the labelled sucrose which appeared in the embryo, labelled glucose and fructose appeared in the reducing sugar pool of the embryo. This could be consistent with the fact that an invertase (optimal activity at *p*H 5·0) can be extracted from this part of the plant at these stages of germination (ref. 2 and Cooper, R. A., and Greenshields, R. N., unpublished results, 1960).

It is proposed in future work to inject other carbon-14 containing compounds into this plant, for example, galactose-^{14}C and raffinose-^{14}C, to determine the fate of these compounds on germination. The raffinose family of oligosaccharides (namely, verbascose, stachyose, raffinose and sucrose) occur in this plant[6] and rapidly disappear on germination, leaving only sucrose in the cotyledons and sucrose, glucose and fructose in the embryo (that is, no galactose)[2]. Thus the fate of the galactose might be determined.

Although effects due to 'cut-respiration'[7] cannot be overcome, because the pattern and changes of the saccharides in untreated plants is the same as in treated plants, the effects due to 'cut-respiration' are probably very small. Furthermore, in the case where injected radioisotopes are translocated to other parts of the plants, for example, sucrose appearing in the embryo after injection in the cotyledons, 'cut-respiration' would not complicate the interpretation of the results.

Thus it can be seen that this technique might provide a useful tool in helping to solve translocation problems and also to indicate possible reactions that occur *in vivo*. Although purely qualitative, the technique indicates lines along which quantitative work might be pursued.

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Effect of pH on the Sensitiveness of Trypsin to Ionizing Radiation

ALTHOUGH oxygen usually increases the sensitivity of biological systems to ionizing radiations, it was found that with the enzymes chymotrypsin[1] and trypsin[2], in dilute aqueous solutions, the presence of air as compared with vacuum diminished the sensitiveness by a factor of about 3. In these experiments the enzyme was dissolved in 10^{-3} *N* hydrochloric acid, in which it was believed to be most stable. We have now, in the case of trypsin, examined the effect of varying the *p*H of the solution in which it is dissolved and find that the effect of oxygen is markedly dependent on the *p*H. Some typical figures of the specific inactivation dose to 220-kV. X-rays are given in Table 1 (in rads/gm./ml.).

Table 1

Solvent	0·1 *N* HCl	0·01 *N* HCl	0·001 *N* HCl	Water
*p*H (approximate)	1	2	3	6
D_{37}/c. in air ($\times 10^{-4}$)	0·3	1·0	1·5	1·0
D_{37}/c. *in vacuo* ($\times 10^{-4}$)	0·25	0·4	0·6	1·0

It can be seen that the difference between the sensitiveness *in vacuo* and in the presence of air is a maximum at about *p*H 3 and diminishes both in more and in less acid solutions. Increasing the chloride ion concentration without changing the *p*H had little effect.

In order to explain this behaviour we must consider the reactions:

$$H + O_2 \rightarrow HO_2 \qquad (1)$$

$$HO_2 \rightarrow H^+ + O_2^- \text{ (}pK\text{ between 2 and 3) (ref. 3)} \qquad (2)$$

$$H_2 \rightarrow H^+ + H \text{ (}pK\text{ between 1 and 3) (ref. 4)} \qquad (3)$$

It can be seen that at $pH > 3$ (approximately) HO_2 will be present under equilibrium conditions mainly as O_2^-, which, like H, is a reducing agent. In so far as we are concerned with oxidation-reduction effects, no great change will be brought about by the presence of oxygen. At $pH < 3$, HO_2, which is an oxidizing agent, will be present in an undissociated form, and it is evident that this is less effective than H or O_2^- in causing inactivation of the enzyme. At still lower pH (~ 2), the formation of H_2^+ begins to be effective, and in this region there will be a competition between H^+ and O_2 for the available H radicals, so that the amount of HO_2 will be diminished. As H_2^+, like H, is a reducing agent, it follows that the effect of oxygen may be expected to be diminished again at low pH.

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BIOLOGY

Survival of Cutaneous Homografts after Transplantation of Lymph Nodes immunized against the Host

Up to now, two main experimental methods have been established to overcome tissue incompatibility in genetically different individuals. One of them is the acquired tolerance in embryogenesis or shortly after birth[1,2], and the other consists in X-ray irradiation of the host with the lethal dose and a life-saving bone-marrow transplantation[3].

In this communication I should like to demonstrate a new approach to overcome tissue incompatibility by influencing the regional lymphatic system with the transplantation of lymph nodes immunized against the host.

I have experimented on rabbits (chinchilla and albino breed) weighing 2,000–2,500 gm. Experiments were performed on three groups within one year. Each group comprised 15 rabbits, five of them being recipients of skin (rabbit *A*, chinchilla), 5 donors of nodes immunized against the recipient (rabbit *B*, albino) and 5 donors of skin (rabbit *C*, albino) (Fig. 1).

From the left ear of the future recipient (*A*) a skin flap 2 cm. × 2 cm. was grafted on the ear of the lymphatic node donor (*B*). One week after the skin graft I extirpated the lymph node from the collecting area of the ear, cut it in thin slices and transplanted it subcutaneously into the base of the recipient's ear (*A*) and, at the same time, grafted the skin of the donor's ear (*C*) on that site.

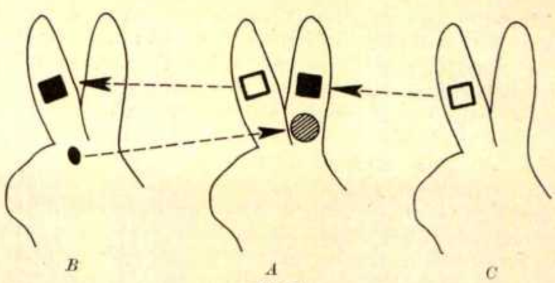

Fig. 1

I have studied the survival of the graft only macroscopically because it differed in colour from the skin of the host. I assessed the result statistically with a *t* test against the control groups in which I simultaneously transplanted on host *A* the node without antibodies against the host and a graft from donor *C*.

The results are summarized in Table 1. In all cases I was able to achieve a statistically extraordinary long survival-rate of the graft. In controls, the graft was rejected between the eighth and the thirteenth day. In the groups after treatment of the regional lymphatic system of the host by antibodies against the host with a transplanted regional lymph node the grafts were rejected in twelve cases between the fifty-fourth and the eighty-sixth day. In three rabbits the graft survived practically permanently.

Table 1. Survival-Rate of Skin Homografts on a Rabbit's Ear

Control group	Influencing of the host with antibodies against the host	Control group	Influencing of the host with antibodies against the host
8	54	12	70
8	58	12	70
9	62	12	79
10	62	12	86
10	63	13	187 (+)
10	67	13	350 (still alive)
11	69	13	746 (still alive)
11	70		
Average : 10·9 ± 0·3	average of the first 12 animals 67·5 ± 2·5		

In one rabbit from the first group, the graft has survived to this day, that is, two years since November 1958 (Fig. 2). In another rabbit from the second group the graft survived macroscopically for six months in perfect condition before the rabbit died of pneumonia. A third graft from the last group is surviving in one-quarter of the original graft for almost one year now in a good condition, that is, since December 1959.

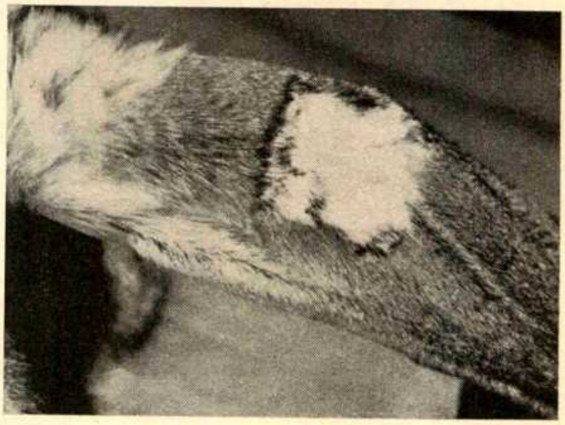

Fig. 2. A homograft two years after the transplantation

The importance of the lymphatic system for achieving transplantation immunity has been differently interpreted in the literature. While the majority of papers stress the changes of the regional lymphatic system after homografts[4] there are also papers categorically denying its importance[5]. In an earlier paper I have attempted to point out the importance of the regional lymphatic system in establishing transplantation immunity. After extirpation of the regional lymphatic system or its destruction by means of *TS* 160 (nitrogen mustard), the survival-rate of the graft could be prolonged for 15–65 days[6].

The results of treatment of the regional lymphatic system with antibodies against the host seem to be important for a new approach to overcome tissue incompatibility.

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A Method of determining Embryonic Orientation in the Hen's Egg

I wish to report a successful attempt at detecting embryonic position in the hen's egg using, so far as it is known, a previously untried technique. It consists of X-raying the incubated egg to determine the skeletal morphology of the embryo. With the egg held in a horizontal position the embryo's longitudinal position can be easily detected. On rotating the egg through a 90° angle, exposing it a second time, and then viewing the X-ray plates in the order of taking, the direction in which the head is turned can be accurately determined.

In this way, not only can the normal orientation of embryos be studied but also the lethal nature or hatchability of certain conventional malpositions about which controversy[1] has arisen in the past, since the technique does not involve the death of embryos.

Fig. 1 shows the normal position adopted by embryos approximately 36 hr. before hatching is completed; the long axis of the embryo coincides with that of the egg and the head and beak are turned to the embryo's right across the breast towards the broad end of the egg and in close proximity to the air cell (clearly defined dark area). In Fig. 2 the longitudinal position of the embryo is reversed so that the head is seen towards the small end of the egg away from the air cell, into which it is normal for the embryo's beak and head to enter during the process of hatching.

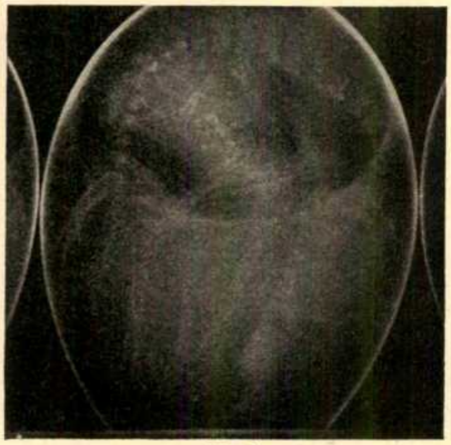

Fig. 1

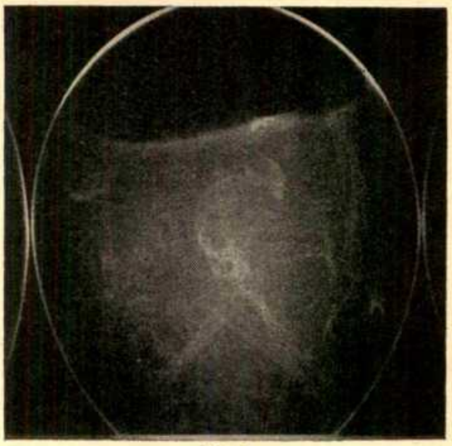

Fig. 2

Detailed results of this work will be reported in the near future; but, for the present, work has shown that (*a*) the head-between-thighs position of malposition I should be regarded as a transitional stage in the normal orientation of embryos to the hatching position rather than a cause of death or failure to hatch, and (*b*) embryos adopting malposition II (head in the small end) or malposition III (head to left) have, respectively, a slightly better than one in two and one in five chance of hatching.

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Effect of Temperature and Grafting on Vegetative Growth Phases of Algerian Ivy

The closely similar English and Algerian ivies, *Hedera helix*, Linn. and *Hedera canariensis*, Willd., do not flower until the juvenile vining type of growth changes into a stiff erect form. This mature type of growth has entire, ovate to lanceloate leaves, and the round stems rarely produce aerial roots. The juvenile trailing stems are somewhat flattened, frequently produce aerial roots, and the leaves are palmately lobed. Both stems and petioles frequently have red pigments. Doorenbos[1] was able to produce many reversions to the juvenile form by grafting the mature fruiting form of English ivy on juvenile two- or three-year seedlings or on juvenile plants grown from cuttings. Plants of adult ivies grown on juvenile understocks for approximately a year outdoors in a lath house in the relatively cool coastal climate of west Los Angeles did not exhibit the reversions described by Doorenbos. Preliminary experiments indicated that mature budded plants on juvenile understocks all reverted to the juvenile form when grown for more than a year at 80° F. but produced few reversions at lower temperatures.

Table 1. GROWTH RESPONSES IN THREE ENVIRONMENTS

Environment	Total plants observed	Plants with adult characters	Plants with juvenile characters	Total No. of shoots	Average shoot length (cm.)
Lath house	66	45	21	155	23·55±1·04
Glasshouse, 60° F.	60	51	9	165	24·45±1·18
Glasshouse, 80° F.	60	0	60	96	25·94±1·52

Closely similar results were obtained in a more extensive experiment started on February 19, 1959, using budded plants of Algerian ivy. The plants were distributed among the three environments on April 20. The glasshouses were air conditioned with automatic controls. On May 4 the natural buds of the stocks were excised to force growth from the inserted buds. On September 14 the plants in both the lath house and the 60° F. glasshouse were predominantly adult and were showing flower buds, but in the 80° F. glasshouse the growth was largely juvenile. The plants were headed back on September 29 to about 6 in. above the bud union. Final results were taken on November 16 and are shown in Table 1. The highest temperature did not increase the length of shoots appreciably, reduced the number of shoots, and promoted reversions to the juvenile type of growth. During this experiment, 26 own-rooted, cutting-grown plants of the mature form of the same clone were kept in each of the same three environments. No changes in type of growth occurred, except that in the glasshouse kept at 80° F. two plants produced shoots with some juvenile characters. On own-rooted mature plants, reversion shoots occasionally appear and are nearly always produced close to the level of the soil.

The amount of mature scion wood initially in the graft combination also influenced the responses. This was demonstrated in an experiment with a clone of Algerian ivy grafted or budded on a juvenile clone of the same species. The plants were started on August 24, 1959, and were placed in the three environments used in the preceding experiments. Buds were excised from the understocks on November 17 in order to force top growth. Growth of the plants in the lath house was slower, but the shoot growth was closely comparable in the two glasshouses. The plants were classified on March 25, 1960. The results are shown in Table 2. These responses show that the greater quantity of the mature type of wood on the scion in grafting diminished the reversions to juvenile growth.

Juvenile scions on mature understocks were also tested. Forty-two grafts, potted on November 17, 1958, were kept in a glasshouse at 60° F. On June 1, 1959, none of the scions had produced any growth which was not fully juvenile. The influence of temperature has been noted in a few other plants. Hudson and Williams[3] noticed that high temperatures favoured the production of the juvenile leaf type on raspberry shoots, and Njoku[4] found a similar relationship in *Ipomoea*, as did Fisher[2] on *Ranunculus*.

Table 2. RESPONSES OF SHOOTS ON BUDDED AND GRAFTED PLANTS IN THREE ENVIRONMENTS

Treatment	80° F.		60° F.		Lath house	
	No. of plants	No. juvenile	No. of plants	No. juvenile	No. of plants	No. juvenile
Budded	73	71	96	14	77	1
Grafted	106	27	101	3	103	0

These experiments confirm the observations of Doorenbos, but also show that reversions to the juvenile form are influenced by temperature and also by the amount of mature tissue present in the graft combinations initially.

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BACTERIOLOGY

Isolation of Lysozyme-soluble Mucopeptides from the Cells Wall of *Escherichia coli*

THE cell walls of the Gram-positive bacteria have been shown by Salton[1] and by Cummins and Harris[2] to consist mainly of polymers, later termed mucopeptides, containing a limited number of amino-acids (usually four), two amino-sugars and sometimes one or more hexoses. Two of the amino-acids, alanine and glutamic acid, are, at least partly, in the D-form. This, together with the occurrence of muramic acid, and sometimes of diaminopimelic acid as well, distinguishes mucopeptides from other types of macromolecule. A further characteristic is sensitivity to lysozyme. The mucopeptides from some bacterial species are completely digested by this enzyme, and all appear to be attacked slightly[3]. The synthesis of mucopeptide in washed suspensions is inhibited by penicillin[4,5].

The cell walls of the Gram-negative bacteria are much more complex (see Salton[6]) and contain a good deal of protein and polysaccharide. However, the presence of mucopeptides similar to those of the Gram-positive bacteria was indicated by the fact that Gram-negative cells could be lysed by treatment with lysozyme[7], and that penicillin produced osmotic fragility in *E. coli*[8]. In addition, when walls of *E. coli* were treated with lysozyme, soluble fragments were recovered containing the characteristic components of mucopeptide, namely, diaminopimelic acid, muramic acid, glucosamine, alanine and glutamic acid[9]. The object of the work to be described was to separate the mucopeptides of *E. coli* sufficiently from protein and polysaccharide to allow its composition and some of its properties to be examined. The procedure finally adopted was as follows.

The bacteria (about 10 gm. dry weight) were broken by shaking with Ballotini beads in a Mickle disintegrator, and the walls sedimented at 10,000*g*. The sediment was suspended in water and heated at 100° C. for 30 min. to destroy any lytic enzymes that might have been present. All further procedures were carried out at room temperature unless otherwise stated. The crude wall preparation was then extracted three times with ethanol/ether (3 : 1) and then with ether. The residue was dried in air, and treated for the reduction and alkylation of disulphide bonds[10] in the protein by suspending the powder in 10 *M* urea

Table 1. MOLAR RATIOS OF AMINO-ACIDS AND HEXOSAMINES IN THE CELL-WALL MUCOPEPTIDES OF *E. coli* AND MICROCOCCI

Strain	Glutamic acid	Alanine	Serine	Glycine	Lysine	**DAP	GM*	Mur.†
E. coli								
W	1·0	1·5	0·13	—	0·19	0·70	0·80	0·51
M	1·0	1·5	0·16	—	0·21	0·67	0·70	0·47
B	1·0	1·5	0·13	—	0·22	0·74	0·70	0·43
K 12	1·0	1·4	0·15	—	0·20	0·63	0·90	0·50
ML 30	1·0	1·5	0·14	—	0·23	0·70	1·0	0·60
Micrococci								
524/*SC*	1·0	1·8	—	3·2	0·6	None	1·0	1·1
H	1·0	3·2–3·7	—	—	0·9–1·2	,,		
Duncan	1·0	2·8	—	6·3	1·9	,,		
11	1·0	1·4	—	0·05	0·5	,,		
Oxford	1·0	1·9	—	4·9	1·0	,,		
M. lysodeikticus	1·0	1·9–2·5	—	1·2–1·6	1·0	,,		

* GM = glucosamine; **DAP = diaminopimelic acid.

† Mur. = muramic acid. Both hexosamines are N-acetylated in *S. aureus* and are presumably so in *E. coli*, although this has not been determined. The molar ratios for coliform mucopeptides were determined as previously described (ref. 13). The values for strains of micrococci are those quoted by Rogers and Perkins (ref. 11).

(50 ml.) containing mercaptoethanol (1 ml.). After several hours, excess iodoacetic acid was added, and the product washed well and digested overnight at 35° C. with pepsin (20 mgm.) in $N/20$ hydrochloric acid. The product still contained more than 50 per cent protein. To remove this, it was extracted three times with 90 per cent formic acid (20 ml.) and twice with 90 per cent phenol (20 ml.). Finally, the residue was extracted three times with 10 ml. of copper ethylenediamine (6 per cent copper hydroxide and 8 per cent ethylenediamine) and washed several times with $N/20$ hydrochloric acid to remove copper. Although some mucopeptide was always lost at this stage, the extraction removed mainly protein and polysaccharide.

After drying with acetone and ether, a white powder was obtained representing about 1 per cent of the bacterial mass, and containing the following major constituents: diaminopimelic acid, lysine, serine, glutamic acid, alanine, glucosamine and muramic acid. Together these accounted for 75–80 per cent of the total material. In addition, chromatography of acid hydrolysates revealed the presence of small amounts of all the other amino-acids characteristic of protein, and these together represented a further 10 per cent. The remainder of the preparation consisted of unidentified carbohydrate.

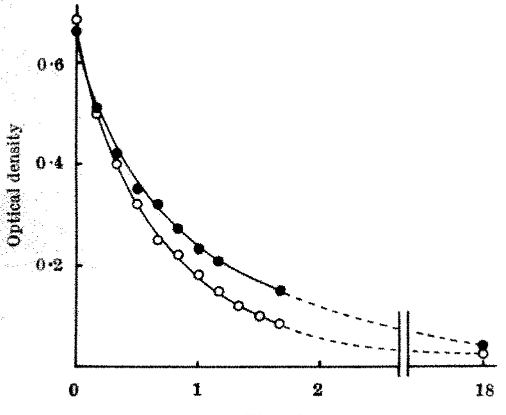

Fig. 1. Digestion of mucopeptides prepared from *E. coli B* and *M. lysodeikticus* by lysozyme. The mucopeptides were suspended at a concentration of 1·5 mgm./ml. in 0·1 *M* phosphate buffer (pH 6·0) and incubated with lysozyme at room temperature. Optical density at 620 mμ was measured at intervals. ○, Values for *M. lysodeikticus* in the presence of 10 μgm./ml. lysozyme; ●, values for *E. coli* mucopeptide, in the presence of 100 μgm./ml. lysozyme. This high concentration of lysozyme was necessary because the mucopeptide from *E. coli* contained an inhibitor of the enzyme apparently introduced during preparation (see text)

To determine whether the method of extraction was likely to damage the mucopeptide, the whole procedure was applied to Gram-positive mucopeptides. The end-product was found to be identical with that obtained by the more conventional methods.

Table 1 shows that the mucopeptides isolated from the five strains of *E. coli* so far examined are virtually identical, and the relative proportions of glutamic acid, alanine, and glucosamine are similar to those found in the mucopeptide of *Staph. aureus* 524. These values, and those for mucopeptides from four other strains of micrococci (see Rogers and Perkins[11]), have been included. It is apparent that, in the latter group, there is much more variability, and an even greater scatter has been found in the walls of lactobacilli[12].

The mucopeptides from *E. coli* were examined for sensitivity to lysozyme, and found to contain an inhibitor of the enzyme probably introduced in the preparation. 1 mgm. of mucopeptide contained sufficient inhibitor to inactivate 10–20 μgm. of lysozyme. However, if sufficient enzyme was added to overcome the inhibiton, there was rapid digestion of the mucopeptide accompanied by a fall in optical density of more than 90 per cent. This is similar to the reduction in optical density found with the mucopeptide of *Micrococcus lysodeikticus* (see Fig. 1).

It thus appears that the cell walls of *E. coli* contain a mucopeptide which, in its composition and its sensitivity to lysozyme, resembles those of the Gram-positive bacteria. The main difference seems to be that whereas the latter have been found to contain either diaminopimelic acid or lysine, the coliform mucopeptides contain both. This conclusion is supported by the fact that lysine increases proportionately with the diaminopimelic acid at each stage of the purification.

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ENTOMOLOGY

A Species of *Tetranychus* Dufour (Acarina) from Uganda

RECENTLY, Baker and Pritchard[1] described certain tetranychoid mites from Africa, among which they listed *Tetranychus cinnabarinus* (Boisd.) and *T. telarius* (L.). Their work embraced a study of preserved material from Namulonge, Uganda, which they ascribed to the former species. The two species mentioned, together with others, have for long been the subject of controversy and have been included in the '*telarius* complex' by authors.

Boudreaux[2] produced evidence, from Nearctic specimens, of a series of constant morphological differences in both males and females whereby the components of the '*telarius* complex' could be separated. Baker and Pritchard applied his principles to their Ethiopian material. Thus the adult female of *T. cinnabarinus* is said to possess lobes on the dorsal integumentary striæ of the hysterosoma which are all semicircular or triangular (Fig. 1, enclosed by circle), whereas those of *T. telarius* are said to be mostly wide (Fig. 1, enclosed by square). Fig. 1 is a photograph of the dorsal integument of the hysterosoma of a typical adult female (feeding stage) of a species of *Tetranychus* commonly found on cotton at Namulonge. With this mite it is possible to follow the series of keys of Baker and Pritchard as far as couplet 9 in the key to species of *Tetranychus*; but at this point neither the name *T. cinnabarinus* nor *T. telarius* can be applied to it since it apparently possesses the integumentary characters of both species. The portion of integument figured displays 80 semicircular or triangular lobes and 30 wide ones; thus neither are they wholly of the former type nor mostly of the latter.

The conformation of the apex of the ædeagus of males of the Namulonge species presents a further anomaly. Baker and Pritchard, again following Boudreaux, described the apex of the ædeagus of of *T. cinnabarinus* as having a somewhat rounded anterior development, an angulate dorsal margin and an axis which forms a slight angle with the axis of the shaft. The drawing which accompanied their description agrees closely with Fig. 2 presented here (type *A*). On the other hand, *T. telarius* is said to have an ædeagus with the anterior development acute, the dorsal margin rounded and the axis parallel to that of the shaft. Fig. 3 represents this type of ædeagus (type *B*). However, Figs. 2 and 3 are photographs of two males of the Namulonge species which were born on the same day of the same female parent, by arrhenotokous parthenogenesis. The female, a typical adult (such as that described above) was isolated on emergence from the deutonymph and the male progeny produced were themselves reared in isolation. Apart from the morphology of the ædeagus, both mites were identical so far as could be observed. Since haploid males are produced in arrhenotoky, it is tempting to assume that ædeagi of types *A* and *B* appear as a result of a simple segregation of the members of a pair of allelic characters in the female, but insufficient material has been examined to establish the ratio in which the two types occur, nor is it possible to state unequivocally that no intermediate form is found.

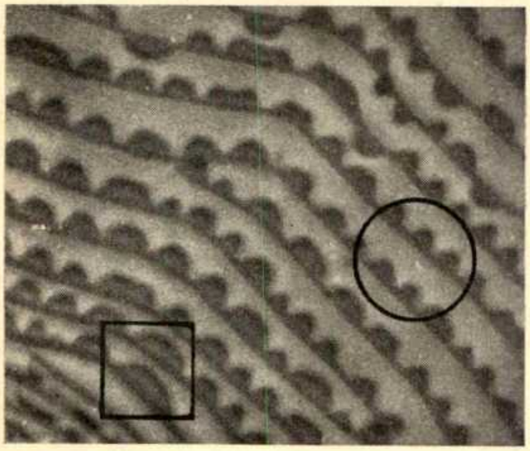

Fig. 1. Dorsal integument of adult female in region of right anterior dorsocentral hysterosomal seta

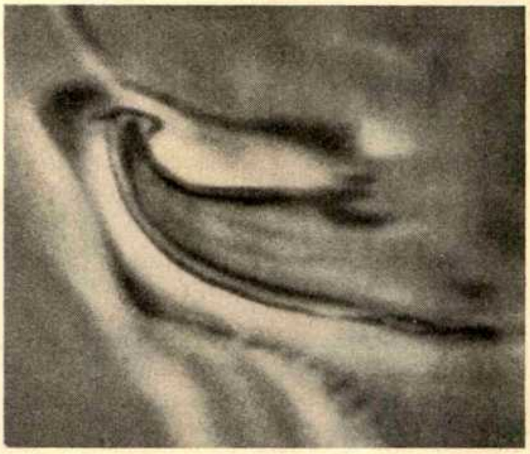

Fig. 2. Lateral view of ædeagus of adult male, type *A*

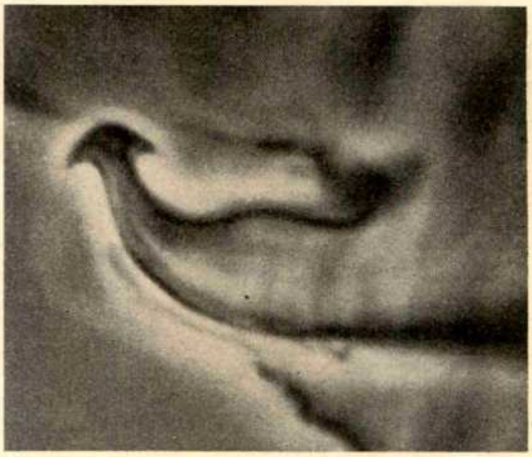

Fig. 3. Lateral view of ædeagus of adult male, type *B*

It is concluded that the means evinced by Boudreaux for the micromorphological separation of the components of the '*telarius* complex' may not be readily applied to all Ethiopian forms before a comprehensive study of the living material has been undertaken.

Specimens figured here were mounted from life in Hoyer's medium, photographed by Zeiss phase-contrast apparatus at 1,000 diameters and afterwards enlarged to 3,750 diameters.

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[1] Baker, E. W., and Pritchard, A. E., *Hilgardia*, **29** (11), 455 (1960).

[2] Boudreaux, H. B., *Ann. Ent. Soc. Amer.*, **49** (1), 43 (1956).

VIROLOGY

Centrosema Mosaic: a Plant Virus Disease transmitted by both Aphids and Plant Bugs

INVESTIGATIONS have been carried out in Papua and New Guinea on a previously undescribed virus disease, *Centrosema* mosaic, which infects several tropical legumes.

The virus is transmitted mechanically, by *Cuscuta campestris* Yuncker, by two aphid species, *Brachycaudus helichrysi* (Kltb.) ? variety *warei* Theob. and *Aphis gossypii* Glov., and by two species of plant bugs of the genus *Nysius* (Table 1).

Table 1. INSECTS CAPABLE OF TRANSMITTING *Centrosema* MOSAIC VIRUS TO *Crotalaria anagyroides* H.B.K.

Insect	Insect form	No. of insects per plant	Proportion of plants infected
Aphididae			
Brachycaudus helichrysi var. *warei*	apterae	10	20/20
Aphis gossypii	apterae	10	6/20
Lygaeidae			
Nysius sp. (green)	winged	20	10/60
Nysius sp. (brown)	winged	20	38/60

It is not usual for a plant virus to be transmitted by both an aphid and a plant bug. *B. helichrysi warei* is able to transmit *Centrosema* mosaic after an access feeding period of 12 min. and remains infectious for 48, but not for 72 hr.; pre-access starvation increases the efficiency of transmission. These facts suggest the virus is transmitted in a non-persistent manner[1]. Winged females, wingless females and first and second instars of *B. helichrysi* var. *warei* are all capable of transmitting the virus.

Though *Aphis gossypii* can transmit the virus under laboratory conditions, it is probably not a natural vector as it has not been recorded feeding on *Crotalaria* spp. or *Centrosema pubescens* Benth., in the field.

Nysius spp. are able to transmit *Centrosema* mosaic to *Crotalaris anagyroides* after an access feeding period of 2 hr. and within a test feeding period of 24 hr.

The virus naturally infects and produces mosaic symptoms on *Centrosema pubescens*, *Crotalaria anagyroides*, *C. goreenis* Guill. and Perr., *C. retusa* L., *C. mucronata* Desv., *Calopogonium mucunoides* Desv. and *Desmodium distortum* (Aubl.) Macbride. The virus can also be mechanically transmitted to *Crotalaris juncea* L., *C. spectabilis* Roth., *C. incana* L., *C. intermedia* Kotschy, *C. lanceolata* E. Mey., and *C. usaramoensis* Baker F., and produces mosaic symptoms in all these hosts. *Stizolobium deeringianum* Bort. exhibits a pale greenish-yellow chlorotic spotting of the first set of trifoliate leaves following inoculations of the first true leaves with infective sap; subsequent leaves show mosaic symptoms which are systemic and persistent. The virus failed to infect *Phaseolus vulgaris* L., *P. mungo* L., *P. aureus* Roxb., *P. lunatus* L., *P. calcaratus* Roxb., *Vigna sinensis* (L.) Savi, *V. unguiculata* (L.) Walp., *Vicia faba* L., *Pisum sativum* L., *Medicago sativa* L., *Canavalia ensiformis* DC., *Datura stramonium* L., *Nicotiana tabacum* L., *N. glutinosa* L., *Lycopersicum esculentum* Mill., *Petunia hybrida* Vilm., *Cucumis sativus* L., or *Chenopodium amaranticolor* Coste and Reyn. following mechanical inoculation. The virus is inactivated after an exposure of 10 min. at 58° C.; at a dilution of 1 in 2,000, and after an exposure of 6 hr. at a room temperature of 28° C.

Further details of these investigations will be published elsewhere.

We acknowledge with appreciation the identification of the insect vectors by Dr. V. F. Eastop of the British Museum, and help given by Dr. M. F. Day of the Commonwealth Scientific and Industrial Research Organization, Canberra.

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[1] Watson, M. A., and Roberts, F. M., *Proc. Roy. Soc.*, B, **127**, 543 (1939).

GENETICS

A Dominant Gene for Renal Adenomas in the Rat

FAMILIAL renal adenomas in the rat have been described in a previous report[1]. They usually occurred as multiple tumours and were bilateral. The tumours were simple cysts, papillary cystadenomas, solid eosinophile adenomas or solid basophile tubular adenomas. They varied in size from barely visible to large growths which included almost the entire kidney (Fig. 1). The material consisted of a number of individuals from a line of Wistar rats maintained in this laboratory, indicating that the condition was genetically controlled.

The breeding of these animals is a laborious process as they have to be opened and inspected to determine the presence of tumours. Males are inspected through a mid-abdominal incision while the kidneys of females are inspected through dorsolateral incisions as they frequently failed to care for their litters after abdominal operations. Furthermore, although

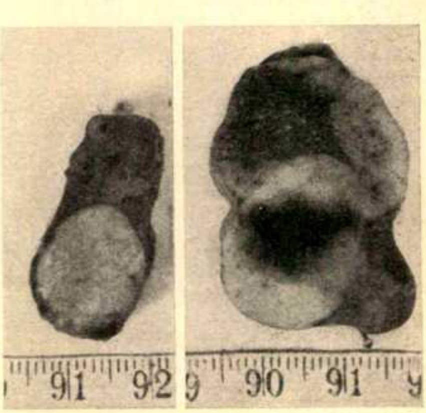

Fig. 1. Longitudinally bisected left and right kidneys of a female with large bilateral tumours

tumours have been found macroscopically in rats as young as five months, they are not usually readily detectable until 10 or 12 months of age, and even then only as small cysts which later develop into solid tumours.

The genetic analysis was begun with a single-tumour male, one of a litter of six, three of which were normal and three had tumours. The mother was tumour-bearing and the father normal. This male was outcrossed to six albino females from a closed laboratory population after having failed to produce any offspring with his three sisters.

A total of 106 F_1 animals was obtained from this outcross, 64 ♀♀ and 42 ♂♂. The inspection and microscopic examination of this first generation have been completed.

A complete post-mortem examination was made on all the F_1 animals, including microscopy of a number of organs, especially the lungs. The types of renal tumours observed are the same as those previously described. In some of them the microscopic picture resembles carcinoma with atypia of the cells and apparently invasion of the surrounding renal tissue. No invasion of the blood vessels has been observed, however, and no metastases have been found in any of the animals in spite of careful examination. The skeleton has also been examined by X-rays in some animals with large tumours, with negative results.

Seventeen of the F_1 animals died too young to be included in the material, leaving a total of 89 F_1 progeny. These were killed or died at ages varying from 19 to 24 months, and the kidneys of all were examined microscopically. All the animals classified as tumour-bearing were verified histologically. One animal macroscopically classified as normal was found to have an incipient tumour on microscopic examination: otherwise there was complete agreement between the macroscopic and the microscopic diagnosis. The results of this classification are presented in Table 1.

Table 1. Histological Diagnosis of Renal Adenoma in F_1 Progeny from a Single Tumour-bearing Male outcrossed to Six Unrelated Females of a Different Strain

	Tumour	Normal	Tumour (per cent)
♀♀	22	31	41·5
♂♂	15	21	41·7
Total	37	52	41 6

This is fairly close approximation to a 1 : 1 ratio.

In order to eliminate the possibility that there might be genetic factors involved in tumour development in the albino strain from which the mothers of the first generation were derived, one of the F_1 tumour males was outcrossed to 20 females of an entirely unrelated strain of hooded rats. Although the examination of all these progeny is not complete, the numbers are sufficiently large to confirm the correctness of the assumption that a single dominant gene determines the development of the tumours. These animals have not yet been examined histologically but the preliminary results of macroscopic examination of 113 progeny from this outcross are: 13 tumours, 19 cysts, 31 doubtful and 50 normal. In this case the sire is known to be a heterozygote, while the original sire of the F_1 might have been a homozygote as it was not possible to trace its ancestry with sufficient accuracy to eliminate the latter possibility.

An F_2 has been obtained by crossing inspected F_1 tumour males to F_1 females which had not yet been inspected. As the tumour females produce few and small litters, only 20 F_2 rats from such matings have so far been inspected at ages of 10–15 months, revealing 4 tumours, 4 cysts, 5 doubtful and 7 negative. This is a somewhat higher ratio of tumour animals than in F_1, but the material is still too small to calculate any ratios, and it is not yet known whether the homozygote is viable.

No correlation has been found between tumour development and sex, nor does there seem to be any influence of number of pregnancies on tumour development in females. The tumours in the F_1 animals were graded for number and size to determine whether there was any correlation between grade and sex; but the results were negative.

Further experiments are being carried out to determine the pathogenesis of the tumours.

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[1] Eker, R., *Acta Path. et Microbiol. Scand.*, 34, 554 (1954).

HISTORY OF SCIENCE

Origins of the Copernican Revolution

The results of the 'Copernican Revolution' have been well described by historians of science. Less has been said about the origins of this revolution; that is, the problems and facts leading Copernicus to a conviction of the physical truth of the Earth's daily rotation and of its annual revolution around the Sun. I have developed a conjecture on this problem which will doubtless prove incorrect when closer study of the relevant sources is made, but which at least directs attention to a natural series of scientific problems, and which is based on Copernicus's own recollections of his early work.

Rheticus, the disciple of Copernicus in the latter's old age, gave in his *Narratio Prima*[1] a list of six principal reasons "why we must abandon the hypotheses of the ancient astronomers". The form of these ensures that they were taken from Copernicus himself. Of the six, the third, fourth and fifth have no surprises, relating to apparent planetary distances, uniform circular motion and economy, respectively. The sixth invokes the rule "that the order and motions of the heavenly spheres agree in an absolute system" and cites the non-observance of this rule by earlier astronomers as a cause of the bad state of the astronomy of the time. The first and second reasons must be quoted in full:

"In the first place, the indisputable precession of the equinoxes, as you have heard, and the change in the obliquity of the ecliptic persuaded my teacher to assume that the motion of the earth could produce most of the appearances in the heavens, or at any rate save them satisfactorily.

"Secondly, the diminution of the eccentricity of the sun is observed, for a similar reason and proportionally, in the eccentricities of the other planets".

These 'reasons' indicate that Copernicus was not exclusively concerned with the problem of short-range predictions of the planetary positions, although this is the only part of his work which has been popularized by historians. Further evidence for a concern with long-term variations comes in the prefatory letter of the *De revolutionibus*. There, first in his list of reasons for thinking of a new method for astronomy, is: "the mathematicians are so unsure of the movements of the sun and moon that they cannot even observe or explain the length of the seasonal year"[2].

It was almost universally believed, from early Islamic times to the sixteenth century, that both the rate of precession (motion of the fixed stars in longitude) and the length of the year were variable. Other parameters of basic astronomical theory were also considered variable (see the foregoing reasons), but I do not take them into account in my present work. A full understanding of these variations was considered necessary for any long-term prediction of the planetary positions, or of eclipses, and for calendar reform. This last was not a trivial task, for one cannot get the true mean of a quantity of unknown variation by averaging over an arbitrary interval. Moreover, the Christian calendar is lunar as well as solar, because of the definition of Easter; this made the problem more delicate. An important part of Copernicus's work was the institution of method in the study of these problems, which had not been treated by Ptolemy. It was for his achievements in these problems that he was called "the restorer of astronomy" by such later astronomers as Clavius. In actual fact, both the precession and the length of the year are constant, and both pseudo-problems died suddenly at the end of Copernicus's century. This is one cause for their neglect by historians. The other is that, from Galileo onwards, debate was centred on the physical and cosmological implications of the Copernican system rather than on the details of his mechanisms.

In my conjecture, I use the 'reasons' as indications of problems, and take a (supposedly) hard fact from the *Commentariolis*[3], written by Copernicus early in his career. This is, that from past records it appears that while the equinoctal year is variable, the sidereal year is constant. This has interesting implications for the choice of the fundamental space-time reference frames for positional astronomy. To explain 'precession', astronomers in the Ptolemaic tradition had assigned a slow but variable motion to the sphere of the fixed stars, a rotation about the pole of the ecliptic. (This was, of course, superimposed on the daily rotation, which was common to all the pre-Copernican theories.) The fixed point of zero celestial longitude was taken at one of the intersections of the great circle of the ecliptic with the extension of the Earth's equatorial plane; and time was measured by the Sun's passage through that point—the equinoctial year.

If, as Copernicus advocated in the *Commentariolis*, we change the definition of the standard year from equinoctal to sidereal (measured by the Sun's return to a given fixed star), an anomaly appears. For then it would be against an irregularly moving space frame (the sphere of fixed stars) that the motions defining uniform time would be measured. This contradicts all principles of harmony in the celestial motions, and such a system cannot serve as a fundamental space-time reference frame for astronomical science. Hence we need a different set of relative motions of the elements of the universe. (I should add here that the justness of the 'sidereal' measure of uniform motion was supported by other slowly varying parameters, or so appeared to Copernicus when he wrote the *Commentariolis*.)

One solution lies in a modification of a theory propounded by Copernicus's teacher, Domenicus Maria de Novara. This was that the Earth's equatorial plane is in motion; it was advanced to explain a supposed change of terrestrial latitudes (measured by the elevation of the pole) since antiquity. Giving the Earth's axis a slow conical motion (the 'Third Motion' of the mature Copernican theory) would free the stellar sphere of its trepidations, and give a harmonious reference-frame with 'sidereal' measures of space and time. It would also explain perfectly another fact reported by Copernicus in the *Commentariolis*: over the ages, a longer equinoctal year had coincided with a slower rate of precession.

But if Copernicus got this far, he then encountered a very serious problem: if this motion occurred, then the direction of the pole of rotation of the heavens, as seen by an observer, would have suffered an impossibly great change since antiquity. My conjecture rests on Copernicus getting this far with the problem, and then taking the only way out which would keep harmony in the heavens: letting the Earth rotate on its axis, while the axis performs the very slow conical motion. With this solution, the problem of the varying direction of the pole disappears. The principle behind the difference between the two solutions is that the finite rotations of a sphere do not commute.

My conjecture thus takes me to the first half of the 'Revolution'. Its extension to the annual revolution is something on which I have no ideas. Even this much bears on Copernicus's conviction of the truth of his theory, in spite of its physical, cosmological and theological difficulties. Sun and circle worship, and mathematical elegance, play their part, but a belief in the rationality and intelligibility of God's heavens may have been crucial.

There are two obvious objections to the principle of this conjecture. The first is that it requires Copernicus to have used two sets of false data (varying length of tropical year, and varying rate of precession), in such a fashion as to get consistent results, and without realizing their systematic connexion. This is easy to explain, for one and the same observational error (misreading the declination of the Sun, and hence misplacing the celestial equator) is responsible for both; orders of magnitude of observation error (5′), and errors of time and precession rate, work out correctly. There is also the fact that Copernicus made no mention of the precession problem in the *Commentariolis*, either in the text or in the introduction. This is not surprising, for at the time of writing it he had not worked out a precise mathematical theory of the 'Third Motion'. It is quite likely that Copernicus was able to work out the heliostatic equant-free planetary models with ease, using the geostatic equant-free models of Ibn Al-Shatir[4] But on those problems which had engaged and persuaded him "in the first place", he would hold back his solutions until they could stand up to detailed criticism.

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[1] In Rosen, E., *Three Copernican Treatises*, second ed., 136 (New York: Dover, and London: Constable, 1959).

[2] Quoted from Kuhn, T., *The Copernican Revolution* (Cambridge: Harvard University Press, 1957), p. 138. Prof. Kuhn suggested there a possible connexion between calendar reform and the heliostatic hypothesis (p. 271), and he has given me generous and valuable assistance in connexion with this study.

[3] Rosen, *op. cit.*, 65.

[4] Kennedy, E. S., and Roberts, Victor, *Isis*, **50**, 227 (1958).

FORTHCOMING EVENTS

(*Meetings marked with an asterisk * are open to the public*)

Monday, March 13

Institution of Mechanical Engineers, Industrial Administration and Engineering Production Group (at 1 Birdcage Walk, Westminster, London, S.W 1), at 10 a.m.—Informal Discussion on "Human Capabilities and Limitations in Industrial Operations".

British Society for the Philosophy of Science (in the Joint Staff Common Room, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Karl Menger : "A Counterpart of Occam's Razor".

Institution of Electrical Engineers (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Town Planning and Electrical Requirements".

Society of Chemical Industry, Agriculture Group (joint meeting with the Surface Activity Group, at 14 Belgrave Square, London, S.W.1), at 5.30 p.m —Mr. G. H. Cashen : "Electric Charges on Clays".

University of London (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Prof. Marcel Simon (University of Strasbourg) : "Origins and Eschatology".* (Further lecture on March 14.)

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. C. E. Engel : "Photography in Medicine" (Cantor Lecture).

Tuesday, March 14

Zoological Society of London (at the Zoological Gardens, Regent's Park, London, N.W.1), at 5 p.m.—Scientific Papers.

Institution of the Rubber Industry, London Section (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 5.30 p m.—"The Union of Rubbers and Plastics", speaker from Polymer (U.K.), Ltd., 7 p.m.—Mr. C. H. Leigh-Dugmore : "Light Microscopy of Black-Loaded Rubber" ; Dr. E. H. Andrews . "Electron Microscopy of Black-Loaded Rubber".

University of London (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. G. D. Sims : "Thermionic Generation".* (Fourth of five lectures on "Generation of Electricity without the Use of Rotating Machinery".)

Institution of Electrical Engineers, Education Discussion Circle (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "New Routes into National Certificate and Technician Courses", opened by Mr. H. W. French.

Wednesday, March 15

Royal Microscopical Society (at Tavistock House South, Tavistock Square, London, W.C.1), at 10.30 a.m.—Symposium on "Applications of Interference Microscopy".

Royal Institute of Chemistry (joint meeting with the Institute of Physics and the Physical Society and the Institute of Biology, in the William Beveridge Hall, Senate House, University of London, W.C.1), at 2 p m —Symposium on "The Soil ; Problems Raised by its Use and Misuse".

Royal Society of Arts (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. S. Tolansky, F.R.S. . "The Folk Lore and History of Diamond".

Royal Meteorological Society (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Dr. W. L. Godson · "Total Ozone and the Middle Stratosphere Over Arctic and Sub-arctic Areas in Winter and Spring".

Royal Society of Medicine, Comparative Medicine Section (at 1 Wimpole Street, London, W.1), at 5 p.m.—Meeting on "Hormonal Control of Fertility in Man and Animals".

Institute of Petroleum (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. J. Groszek and Mr. D. J. Palmer . "Surface-Active Compounds in Lubricating Oils".

Institution of Electrical Engineers, Supply Section (at Savoy Place, London, W.C.2), at 5.30 p m.—Discussion on "Magneto-Hydrodynamic Generation of Electricity" opened by Dr. P. L. Davies and Dr. D. J. Harris.

Institution of Electrical Engineers, London Graduate and Student Section (joint meeting with the Graduate and Students Sections of the Institutions of Civil and Mechanical Engineers, at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 6.30 p.m.—Prof. Sir Harrie Massey, F.R.S. · "Space Research".

Society of Instrument Technology, Data Processing Section (at 26 Portland Place, London, W.1), at 7 p.m.—Symposium on "Burst Slug Detection in Nuclear Power Stations".

British Psychological Society, Social Psychology Section (in Room 425, Birkbeck College, Malet Street, London, W.C.1), at 8 p.m —Mr Joseph Trenaman : "Television and Political Images".

Thursday, March 16

Institute of Physics and the Physical Society, Optical Group (at 47 Belgrave Square, London, S.W.1), at 2.30 p.m.—Annual General Meeting.

Royal Society (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Meeting for the Election of Fellows. 4.30 p.m.—Mr. F. P. Bretherton and Lord Rothschild, F.R.S. . "Rheotaxis of Spermatozoa" ; Mr. D. J. Crisp and Mr. A. J. Southward : "Different Types of Cirral Activity of Barnacles with special reference to Sessile Species".

London Mathematical Society (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. F. R. Keogh : "On a Problem of Hardy and Littlewood concerning Rearrangements".

British Institution of Radio Engineers, Electro-Acoustics Group (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. S. Welldon : "Transistorized Tape Recorders".

Institution of Electrical Engineers, Utilization Section (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. D. S. Farr, Mr. H. C. Hall and Dr. A. L. Williams : "A Dynamic Model for Studying the Behaviour of the Overhead Equipment used in Electric Railway Traction".

Society of Chemical Industry, Road and Building Materials Group (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. J. A. Reis, Miss Barbara E. Sabey and Mr. J. G. James : "White Line Road-markings and Light Coloured Road Surfaces".

Association of Clinical Biochemists (in the Lecture Theatre, Royal Free Hospital Medical School, Hunter Street, London, W.C.1), at 7.30 p.m.—Prof. H. B. Collier · "Recent Advances in Red Cell Chemistry".

Chemical Society (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Prof. A. N. Terenin : "Change Transfer in Organic Solids, Induced by Light" (Centenary Lecture).

Royal Society of Tropical Medicine and Hygiene (at the Royal Army Medical College, Millbank, London, S.W.1), at 7.30 p.m. —Laboratory Meeting. Chairman : Sir William MacArthur, K C.B.

Friday, March 17

Society for Water Treatment and Examination (in the Library, Royal Society of Health, 90 Buckingham Palace Road, London, S.W.1), at 9.30 a m.—Annual General Meeting. 10.30 a.m.—Symposium on "Application of Laboratory and Pilot Plant Studies to Full Scale Water and Effluent Treatment".

British Psychological Society, Occupational Psychology Section (in the Department of Psychology, Birkbeck College, Malet Street, London, W.C.1), at 1 p m.—Mr. R. B. Buzzard : "An Approach to the Classification of Working Environments".

Institute of Rural Life at Home and Overseas (at Friends International Centre, 32 Tavistock Square, London, W.C.1), at 1.15 p.m.—Mr. Peter du Sautoy : "The Ghana Community Development Programme".

Institute of Navigation (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Captain V. A. M. Hunt . "The Long-Term Plan for Air Traffic Control".

Society of Chemical Industry, London Section (joint meeting with the Fine Chemicals Group, at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. L. M. Jackman : "The Use of Nuclear Magnetic Resonance in the Determination of Chemical Structure".

Royal Institution (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. R. S. Nyholm, F.R S. : "Magnetism and Chemical Architecture".

Saturday, March 18

Nutrition Society (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 10.30 a.m. —Symposium on "The Place of Food Science and Technology in the Campaign against Malnutrition".

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned :

British Oil and Cake Mills Research Fellow (graduate in agriculture, agricultural science or pure science, preferably with honours) in the Department of Agriculture—The Professor of Agriculture, The University, Leeds 2 (March 20).

Senior Lecturer in Metallurgy—The Registrar, The University, Nottingham (March 24).

Vice-Principal (with high academic qualifications and teaching experience at a high level)—The Clerk to the Governors, Chelsea College of Science and Technology, London, S.W.3 (March 24).

Lecturer in Applied Mathematics ; a Lecturer or Assistant Lecturer in Pure Mathematics ; and a Lecturer or Assistant Lecturer in Pure or Applied Mathematics—The Registrar, The University, Hull (March 29).

Assistant Curator (with a university degree in biology and the Diploma of the Museums Association) in Natural History at the St. Albans City Museum—The Town Clerk, 38 St. Peter's Street, St. Albans, Herts (March 31).

Lecturer or Assistant Lecturer in the Department of Genetics—The Assistant Registrar (Science), The University, Edgbaston, Birmingham 15 (March 31).

Research Fellow in the Department of Geology—The Registrar, The University, Edgbaston, Birmingham 15 (March 31).

University Demonstrator (with an honours degree in an appropriate science) in Agricultural Entomology—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (March 31).

Head (graduate, with research experience in such fields as genetics, avian physiology, nutrition or embryology, and preferably experience in any field of poultry science) of the Poultry Research Section

—The Secretary, Wye College (University of London), near Ashford, Kent (April 1).

ASSISTANT LECTURER OR LECTURER IN ORGANIC CHEMISTRY at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 8).

ASSISTANT LECTURER IN CROP PRODUCTION—The Secretary, Wye College (University of London), near Ashford, Kent (April 15).

CHAIR OF PHYSICS—The Registrar, The University, Sheffield (April 15).

LECTURER (honours graduate) IN PHYSICS—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (April 15).

LECTURER IN MICROBIOLOGY; and an ASSISTANT LECTURER IN PLANT ECOLOGY IN THE DEPARTMENT OF BOTANY—The Secretary, University College, Gower Street, London, W C.1 (April 15).

LECTURER (preferably with experience in techniques dealing with root infecting fungi) IN BOTANY in the School of General Studies, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 15).

LECTURER or ASSISTANT IN PHYSIOLOGY—The Secretary of University Court, The University, Glasgow (April 29).

RESEARCH PROFESSOR IN NUCLEAR PHYSICS at the Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, May 1).

ASSISTANT TRIALS OFFICERS (preferably with practical experience in crop production) at Leeds University Farm, Headley Hall, Yorks, and at Seale-Hayne Agricultural College, Newton Abbot, Devon—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

PROFESSORS IN NUCLEAR PHYSICS, PHYSICAL CHEMISTRY, and HYDRAULIC ENGINEERING at the University of Peshawar—The Ministry of Labour (E.9), 26–28 King Street, London, S.W.1.

SENIOR LECTURER IN PHARMACOGNOSY; a SENIOR LECTURER IN PHARMACEUTICS; a SENIOR LECTURER IN PHARMACOLOGY; a LECTURER IN PHARMACEUTICAL CHEMISTRY; a LECTURER IN PHARMACOGNOSY; a LECTURER IN PHARMACEUTICS; and a LECTURER IN PHARMACOLOGY—The Registrar (Department D), Bradford Institute of Technology, Bradford 7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Privy Council. Medical Research Council Monitoring Report Series No. 1 Assay of Strontium-90 in Human Bone in the United Kingdom—Further Results for 1959. Pp. v+18. (London: H.M. Stationery Office, 1960.) 1*s*. 6*d*. net. [212

Institute of Personnel Management. Occasional Papers, No. 16: Man and his Machines—Automation and Computation, their Origins and Effects. By Sir Ben Lockspeiser. Pp. 12. (London: Institute of Personnel Management, 1960.) 2*s*. 6*d*. [212

Department of Scientific and Industrial Research. Summary of Progress of the Geological Survey of Great Britain and the Museum of Practical Geology for the year 1959, with Report of the Geological Survey Board. Pp. iii+76. (London: H.M Stationery Office, 1960.) 5*s*. net. [212

Ministry of Housing and Local Government. River Severn Basin: Hydrological Survey (Hydrometric Area No. 54). Pp. iv+135. (London: H.M Stationery Office, 1960.) 17*s*. 6*d*. net. [212

Catalogue of Reproductions of Celestial Photographs published by the Royal Astronomical Society. Pp. 39. (London: Royal Astronomical Society, 1960.) [212

Report of the Woolfson Foundation, 1955–1960. Pp. 39 (8 plates). (London: The Woolfson Foundation, 1960.) [612

Technical Books-in-Print, Vol. 1, No 8 Physics and Mathematics (September 1960). Edited by W. G. Smith, F L.A. Pp. 60. (London. Hansom Books, Ltd., 1960.) 6*d*. [612

The British Council. Annual Report, 1959–60. Pp. vi+110+12 plates. (London: The British Council, 1960.) [612

Titration in Non-Aqueous Solvents. By A. H. Beckett and E. H. Tinley. Third edition. Pp. iv+56. (Poole: British Drug Houses, Ltd., 1960.) [612

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Annual Report of the Calcutta School of Medicine and the Carmichael Hospital for Tropical Diseases, 1958–1959. Pp. i+126. (Calcutta: Calcutta School of Tropical Medicine, 1960.) [212

Canada: Department of Mines and Technical Surveys. Publications of the Dominion Astrophysical Observatory, Victoria, B.C. Vol. 11, No. 9: The Spectroscopic Orbit of θ^2 Tauri. By E. G. Ebbighausen. Pp. 235–245. Vol. 11, No. 10: The Spectroscopic Orbit of γ Orionis. By E. G. Ebbighausen and R. M. Petrie. Pp. 247–252. Vol. 11, No. 11 Shock Waves in the Atmosphere of a Long-Period Variable. By G. J. Odgers and R. S. Kushwaha. Pp. 253–258. Vol. 11, No 12: The Spectroscopic Binary H. D. 95638. By R. M. Petrie. Pp. 259–264 Vol. 11, No. 13: The Orbit of the Spectroscopic Binary ω Ursae Majoris. By E. G. Ebbighausen. Pp. 265–275. Vol. 11, No. 14: The Spectroscopic Orbit of β Trianguli. By E. G. Ebbighausen. Pp. 277–282. Vol. 11, No. 15. A Line Intensity Study of the Spectrum of H D. 188001, 9 Sagittae. By Anne B. Underhill. Pp. 283–306. (Ottawa: Queen's Printer, 1960.) [212

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AN EDUCATION FOR LIFE

ONE cannot help but feel a pang of sympathy for the youth of Britain to-day, gazing wide-eyed at the promised good (or goods) of our technological era. For it seems that the promised good may conceal and nurture a stifling evil.

In a report arising from the annual conference of the British Association for Commercial and Industrial Education*, critical surveys of recent policy recommendations in the field of education are presented. A final article summarizes these very aptly. It also proffers the happy conjecture that in A.D. 3000 posterity, on finding the Crowther, Albemarle, Carr, Jackson, McMeeking, Arnold and Anderson Reports in a casket, will experience a positive reaction to the energy and vision that was contributed to reassessing education for British youth.

The need for all this drastic reform springs from "a pressure quite outside the scope of public control, for its comes from outside . . . relates to the position of Great Britain among her trade competitors in other countries and the influence upon this of the system of higher education in this country". The old order of education whereby men were equipped with a "proper background" must yield to the new scheme of "indoctrinating them with certain forms of knowledge". They must now train to be of direct use to improve industrial processes and products. The knell of industry sounds ever shriller. Specialize, 'technologize' or perish. Hence, happily or otherwise, the country must eddy along in this sweeping technological turbulence.

In the teeth of the gale of change, perhaps we should try to preserve some degree of self-control, however, and not throw out existing good as so much ballast. To persuade the keenest minds of our generation into a technological career as the glowing imperative, could result in irrecoverable loss, particularly when it conflicts with natural tendencies in other directions. It is not fair. Some personalities, some minds, are suited to such a career, others not. Almost platitudinous, it may be said ; but then a platitude has been defined as a self-evident fact on which nobody acts.

To get the cream of British minds recruited to serve technology, we eulogize the virtues of applied science—'it performs everything by the surest rules and demonstrations'—in the Baconian sense. But we tend to gloss over the pedestrian qualities which are inescapably there. We show that the job of an applied scientist is to evaluate coefficients of the differential equations which physicists and mathematicians have formulated long before. This job is done using experiment, curve-fitting, statistical manipulation and computers when the mathematics is not amenable to implicit, analytical solutions. Useful and skilled activity, to be sure, but we may tend to glorify it as a creative process of the highest order, forgetting that what one technologist learns to manipulate, another can. Our unbridled enthusiasm may lead us to believe that training a man to be a directly useful component in industry is the *raison d'être* of education. Perhaps we should be a trifle more restrained and tell those brighter youths, who wish to do creative work, that a career in technology is very often a consuming, grim grind and a quest for half-truths.

The educational system of a country is an expression of its national ideal. Despite the obloquy levelled against the British system from inside, one cannot deny that it has produced men eminently civilized and competent. Turn to the international arena, where the unfortunate East–West technocracies clash, and note the undisguised, fierce barbarism on one hand and the poorly disguised sentimentality and emasculation on the other. Then look at Europe, with Britain, and recognize quality. In the past, as now still, British education served to produce men who were literate, able to reflect, to judge and to converse. They understood the broad sweep of things. In a recent article*, Prof. M. W. Thring adduces that to-day "we have the much more concrete problem of making scientific laws seem exciting and interesting to a large proportion of our youth". What a pity that we must make these laws only seem so. Do we fall into inadvertent deception to serve the great-god, 'Technology' ? Must the "combustible material" in the minds of our youth (Prof. Thring's imagery) be made a burnt-offering to the avaricious deity ?

The British have always shown a proclivity to retain what they have proved good over centuries. Now we are beginning to distrust our judgment. Trans-Atlantic fever for uprooting itself fascinates : "In America industrial management will very often scrap plant just because something newer has come along. . . . We keep old plant because we are fond of it. . . ." We gaze bewitched at the Kremlin and Peking ; the Labour University at Kiangsi has 50,000 students and there are 1,065 such institutions in China. What a paradisial plethora of technologists there must be. Instead of being naively deceived as to what constitutes quality, we should pause and consider where the fount of fundamental scientific ideas springs from, and then note the educational environment which has given rise to these meditative, inspired minds.

We have indeed a grave responsibility to British youth. Let not the national ideal be to turn out mass-produced stereotypes who cannot converse or use language properly except as the jargon of their particular narrow occupation, who seek only to speak of facts, dull though these may be, who cannot stand up to dialectical encounter, who show distinct discomfort when ideas, feelings and imagination are introduced. Let us respect the individual. Instead of forcing or trapping the talented into a technological constraint, let us rather guide them wisely toward, and allow them to flourish in, the environment to which they naturally would incline.

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* Policy in Perspective Report of the BACIE Annual Conference held at Cambridge, 22–24 September, 1960. Pp. 126. (London: British Association for Commercial and Industrial Education, 1960.) 10s.

* *The Advancement of Science*, Vol. 17, No. 69; January 1961. Pp. 456–460. (London: British Association for the Advancement of Science, 1961.) 7s. 6d.

Amid the frenzied pressures of this decade, it seems appropriate to refer to the Earl Grey Memorial Lecture (*Today and Yesterday*) delivered by G. M. Young. He felt that the duty of a State is to build up a nation which, in the Aristotelian sense of the educated, the civilized mind, knows what questions to ask and what answers to be satisfied with. Before the passage from adolescence to manhood is complete, there should be implanted in the child an appreciation of domestic coherence, sense of stock, significance of the hearth and of the society which goes on and on for ever and is by nature the home. He should be guided in his education from the dogmatic stage, where there is passive acceptance, to the dialectical, where the infinite variety of interpretations of life is grasped. Here he, who is trained in a purely technical sense, often fails abysmally. Imbued with the rules and procedures of his precious doxology, anything outside his little province is scarcely credible, if at all comprehensible.

Mr. Young believes that people cannot be educated at a much greater rate than their families are being civilized. No doubt, however, if proper education becomes more and more available, greater numbers will emerge from homes where true intellectual sympathy reigns. With the canons of science and the art of reasoning so generally understood, the relationship between the learned and the ignorant would be one of confidence. So, even in the new order, Britain will preserve that sense or instinct of continuity; each generation being duly trained to step into the succession when the time comes. In effect, Britain would thus rear a democracy which was also an aristocracy. If no longer a great power, let us strive to be a good one.

So, beset by the dominant influences of the world to-day—fierce, passionate despotism on one side and sick, surrealistic anarchy on the other—Britain must eschew imitation. Instead, may the good bequeathed by the experience of ancestry be recognized and retained. May it continue, with Wordsworthian sentiment, "To make the gift of life more valuable and the men more worthy of the gift".

INTRODUCTION TO PLANT ANATOMY

Anatomy of Seed Plants

By Prof. Katherine Esau. Pp. xvi+376. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1960.) 56*s*. net.

IT is essential for all students of botany to make themselves well informed about the structure of plants. This applies to those who will ultimately become specialists as well as to those whose knowledge of botany remains more general. In spite of the importance of plant anatomy it is remarkable how seldom we find that a student's knowledge of this branch of botany reaches the same standard as that which he attains in others. This situation probably arises, at least in part, because there are so few botanists who are actively engaged on research into the classical aspects of plant histology, and teachers of the subject tend to be purveyors of second-hand information and lack the inspiration that can come only to those fighting in the front line to acquire new knowledge. Prof. Esau has, for many years, been actively engaged on plant anatomical research. She can therefore speak as one having authority based on first-hand experience, and, in the book under review, she does so in clear and simple language. She illustrates what she has to say with line drawings, photomicrographs and a few electron micrographs. These illustrations, of which only a few have been supplied by other authors, are well chosen and of quite outstanding quality. These facts will cause no surprise to the many students and research workers who are already familiar with Prof. Esau's larger text-book entitled *Plant Anatomy* (John Wiley and Sons Inc., 1953), which has come to be accepted as one of the best books of its kind in the English language. The high price of the larger book has, however, undoubtedly prevented it from being as widely used among impecunious students as it deserves to be, and the shortened version which Prof. Esau has now produced, although the price is still fairly high, can be sure of a ready welcome. Indeed, it is to be hoped that the carefully selected anatomical information which it contains will be mastered by all students of botany and that it will serve as the terra firma of fundamental knowledge on which their subsequent studies may be securely grounded.

The book is divided into 22 chapters. The subject is treated mainly from the developmental point of view and deals not only with the cells and tissues of adult organs, including those of flowers, fruits and seeds, but also treats the plant at all stages from the embryo to maturity. There are indications of how the structure of the plant is related to ecological conditions, to taxonomy and to physiology. There is little to be found to criticize in a book of such undoubted excellence and that is so well produced and printed, but the reviewer feels that the relationship of structure to physiological function could be usefully expanded. For example, where Prof. Esau is discussing phloem, on the structure of which she is indeed a leading authority, one is intrigued to read that the nature of the protoplasts of sieve-tube elements and of the connecting strands in sieve areas must be understood before we can reach a satisfactory explanation of how food is translocated. It is also interesting to read that "the known characters of the protoplast do not wholly support any of the hypotheses advanced so far with regard to the (translocation) mechanism". Having had his appetite thus whetted, the reader may well feel that he would like to know more about Prof. Esau's views on this all-important topic; but perhaps the explanation is that she has thought it wise not to commit herself too far in an elementary text-book.

Selected bibliographies are given at the ends of the chapters, and the book contains an excellent and all-important glossary of anatomical terms. Such glossaries are very much needed, especially in these days, when there is often a lack of precision in the meaning of terms, when the language employed by wood anatomists is not always familiar to other anatomists, when pharmacognoscists sometimes tend to use terms in senses that do not always agree with those used in botany departments, and when electron microscopists and classical anatomists do not mean quite the same thing when they use a term as familiar as 'primary wall'. Most of the terms that Prof. Esau has adopted should be readily accepted by all, but botanists in Great Britain may be surprised to find the white- and yellow-coloured tissue in the rind of an orange referred to as the 'albedo' and 'flavedo' respectively.

Prof. Esau lays special emphasis on the unity and interdependence of the parts of a plant as revealed by developmental studies. This unity is further stressed in the introduction to this book where we read that: "The separation of the plant into organs can be made only approximately. It is impossible, for example, to draw a clear demarcation between the shoot and root and between stem and leaf, and the flower in many ways resembles the vegetative shoot. The internal structures, similarly, are not sharply delimited, and the categories of cells and tissues show much intergrading". How true this all is, and how helpful it would be if it were more generally recognized among botanists that Nature abhors distinct lines of demarcation. The classification of cells and tissues, like that of the plants themselves, is by no means easy.

In this book the author successfully vitalizes the whole subject, and the reader can scarcely fail to realize at the outset that he is being asked to consider cells and tissues in living organisms and not in dead corpses. It is to be hoped that this vital approach will stimulate a widespread desire among students to know more about this important aspect of plant science which is often so needlessly thought of as being dull.

C. R. METCALFE

AN ANATOMY OF GRASSES

Anatomy of the Monocotyledons

By C. R. Metcalfe. Vol. 1: Gramineae. Pp. lxi+731. (Oxford: Clarendon Press; London: Oxford University Press, 1960.) 84*s*. net.

METCALFE AND CHALK is a name as familiar to the botanist as Mrs. Beeton is to the cook and, like Mrs. Beeton, it contains a vast amount of information largely derived from first-hand observation.

The present work is another monument to the skill and indefatigable industry of Dr. Metcalfe, who once again puts plant anatomists and taxonomists in his debt.

The main part of this volume consists of a systemati account of the anatomy of 206 genera and 413 species of grasses which have been investigated by Dr. Metcalfe in the past ten years; to this is added information obtained from the literature about a further 139 genera, so that something of the order of half the genera in the family are considered. Since the genera of grasses, like those of most other families of flowering plants, do not fall readily into a linear sequence, an alphabetical order has, very sensibly, been adopted for this 'histological dictionary' section of the book. The bamboos are regarded as sufficiently distinct in their leaf anatomy to be treated separately after the grasses belonging to other tribes have been discussed. The merit of this arrangement is perhaps open to question, since there are genera, such as *Anomochloa* and *Pariana*, which have many bambusoid features, and the distinction between the panicoid and festucoid types of leaf seems to be almost as clear-cut as that between these types and the bamboos. There might therefore have been something to be said in favour of a completely alphabetical arrangement; this is, however, a minor point.

The introductory chapters on the general morphology of the grass plant and on anatomical characters and their value to the taxonomist provide both a basis for the understanding of the dictionary and an admirable summary and discussion of the detailed information. Dr. Melville is himself very cautious in considering the implications of his anatomical observations for taxonomy, but to one whose knowledge of grass taxonomy is slight and of anatomy even slighter they seem to be of the first importance. Some of them have already been incorporated in taxonomic works by Dr. C. E. Hubbard and others; no doubt the remainder will be speedily utilized.

In this connexion a particularly valuable service is performed in directing attention to genera such as *Danthonia*, which are anatomically heterogeneous, as the desirability of a re-examination of their floral morphology and an investigation of their chromosomes is clearly indicated.

In conclusion, mention must be made of two other excellent features of this book. One is the account of the fairly simple but not generally known methods used in preparing both fresh and dried material for microscopic examination, and the other is the beautiful drawings of the main types of structures described in the text.

One is left with an enhanced feeling of admiration for both the Gramineae and Dr. Metcalfe, and a pleasurable anticipation of further volumes on other families of Monocotyledons.

T. G. TUTIN

SURVEYS IN APPLIED MATHEMATICS

Elasticity and Plasticity

By Prof. J. N. Goodier and Prof. P. G. Hodge. (Surveys in Applied Mathematics, Vol. 1.) Pp. ix+152. 50*s*. net.

Dynamics and Nonlinear Mechanics

By E. Leimanis and N. Minorsky. (Surveys in Applied Mathematics, Vol. 2.) Pp. xii+206. 62*s*. net.

Mathematical Aspects of Subsonic and Transonic Gas Dynamics

By Lipman Bers. (Surveys in Applied Mathematics, Vol. 3.) Pp. xv+164. 62*s*. net.

(New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.)

THE great flood of papers in applied mathematics which now pours endlessly through scientific journals from individuals and research groups throughout the world makes it impossible for the practising mathematician to keep fully abreast with new developments, except in some special field in which he is himself working. It follows that surveys of recent work written by authorities in their respective subjects and intended for the mathematically literate reader who is not a specialist in that subject can be of great value. In particular, they serve to direct attention to ideas and methods developed in one subject which may have useful applications in another. The present volumes are good examples of such surveys. A particularly valuable feature of them is the special attention their authors have paid to recent Russian publications. Indeed, one of the most notable features of these surveys is the great volume and importance of the Russian contributions to most of the subjects discussed.

In the first part of the first volume of the series, Prof. Goodier describes some recent advances in the mathematical theory of elasticity. The most significant of these advances undoubtedly springs from the work of Muskhelishvili, and a great deal of the survey is devoted to his work. His early work on complex potential theory, leading to elegant and comprehensive methods of solving plane problems with prescribed boundary forces or prescribed boundary displacements, is briefly described. This is followed by a rather more detailed, though still condensed, discussion of Muskhelishvili's fundamental work in the theory of complex singular integral equations and the associated Hilbert problem. The solution of this problem using Plemelj's theorem provides a remarkably powerful method of solving mixed boundary value problems. The fundamental theory has of course been available for some years to English readers in J. R. M. Radok's translations of Muskhelishvili's two classical treatises, but there is also a considerable Russian literature on detailed applications to specific problems; the present survey gives a most useful summary of these applications. Other topics briefly dealt with include anisotropic elasticity, thermal stresses, contact problems and wave propagation. The theory of finite deformations is referred to but not described, since an excellent recent account of this is to be found in Green and Zerna's book.

The other part of Volume 1 is a discussion by Prof. Hodge on the present state of the mathematical theory of plasticity. Here the emphasis is rightly placed on the derivation of the fundamental principles and equations of the theory, rather than on methods of solution of specific problems, though one problem, that of a simply supported plate with various types of plasticity, is dealt with in detail as an illustration of the methods at present available; references are also given to a number of other problems. There is a very readable introduction to the general theory, and the postulated properties, such as perfect plasticity, piece-wise linear plasticity, kinematic and isotropic strain-hardening, etc., of various ideal substances are clearly defined. The plastic-flow laws are derived, and the inadequacy of any deformation theory for discussing many problems is emphasized. A short chapter is devoted to Russian contributions, but the very brief descriptions of these prove insufficient, at least to me, to indicate their value and significance.

The second volume of the series is also divided, the first part by E. Leimanis being mainly a discussion of the two celebrated problems in classical dynamics, (i) the motion of a heavy rigid body about a point and (ii) the three-body problem. There is also a chapter on exterior ballistics. Many of the investigations considered can scarcely be described as recent. The solutions of the special cases of the rigid body problem associated with the names of Euler, Lagrange and Kovalevskaya are discussed in some detail, and the investigations of Poincaré, Hess, Schiff and others on the existence of other solvable special cases are described. Almost all recent work relates to special cases, and no significant advance in the theory of the general motion is reported. The situation is not very different for the three-body problem. Little progress towards a general understanding of the problem can be discerned since Sundman's solution of 1912. Indeed, Prof. Leimanis devotes a considerable section to showing that Vernić's recent attempts (1952) to extend the validity of Sundman's solution to triple collisions have not been successful. More satisfactory progress has been made in studies of periodic and asymptotic solutions of both the restricted and the general three-body problem, and Merman's work in recent Russian papers on the problem of capture seems to be perhaps the most significant of recent advances.

In the second part an excellent introduction to the theory of non-linear oscillations is given by N. Minorsky, beginning with and largely based on van der Pol's equation $\ddot{x} + \mu(x^2 - 1)\dot{x} + x = 0$. The important topological concepts introduced by Poincaré, the identification of singular points in the phase plane with positions of equilibrium, limit cycles with stationary motions and so on, are adequately explained, and various approximate methods of solution of the equation $\ddot{x} + x = \mu f(x,\dot{x})$, where μ is small, are described. The radical change in character of the problem when μ is not small is emphasized, and the different methods of approach which have been tried in this case are reviewed. The important work of Cartwright and Littlewood on the conditions for existence of a periodic solution of van der Pol's equation is mentioned, but does not seem to have led to a practical method of solution. Another method of approach is the discontinuous theory of relaxations, analogous to the theory of shocks. This has been developed mainly in the U.S.S.R. by Chaikin, Lochakov and others, and seems at present to offer the most practical tool for applied problems.

In the third volume of the series Prof. Lipman Bers discusses certain mathematical aspects of subsonic and transonic gas-flow theory. He deals exclusively with steady irrotational flows and almost exclusively with two-dimensional problems. He places more emphasis on mathematical concepts and techniques than on specific physical problems, but gives some indication of recent practical methods and results. He devotes much of his discussion to the questions of existence and uniqueness of solution of various problems. Since the governing equations of compressible flow are non-linear and of mixed type, these questions involve some highly sophisticated mathematical arguments, and only recently has much progress been made in finding answers to them. Chapter 1 is a good introduction to the subject and describes the various approximations and modifications of the potential flow equation. Chapter 2 is concerned with the general theory of elliptic equations, quasi-conformal mapping and the theory of pseudo-analytic functions to which the author has himself made important contributions. Chapter 3 applies the results of this theory to subsonic flows. Tricomi's theory of mixed elliptic-hyperbolic equations is discussed in Chapter 4 and applications to transonic flow are given in Chapter 5. A short appendix is added on numerical methods. This volume is by no means easy to read, but it contains much of interest and value to the applied mathematician.

In conclusion, it may be said that these three volumes, though somewhat uneven in their degree of mathematical sophistication, achieve their purpose of providing a good overall picture of the present state of certain fields of study. They are well produced, and few misprints appear to have crept in, though some were noticed in the third volume.

A. T. Price

NEBULÆ, GALAXIES AND COSMOLOGY

Rival Theories of Cosmology

A Symposium and Discussion of Modern Theories of the Structure of the Universe. By Prof. H. Bondi, Dr. W. B. Bonnor, Dr. R. A. Lyttleton and Dr. G. J. Whitrow. Pp. xii + 61 + 12 plates. (London and New York: Oxford University Press, 1960.) 9s. 6d. net.

l'Exploration des Galaxies Voisines par les Méthodes Optiques et Radio-Électriques

Par Dr. Gérard de Vaucouleurs. (Collection "Évolution des Sciences", No. 13.) Pp. 156 + 18 planches. (Paris: Masson et Cie., 1958.) 1,600 francs.

Le Nebulose e gli Universi-Isole

Da Giorgio Abetti e Margherita Hack. (Biblioteca di Cultura Scientifica, No. 62.) Pp. x + 354. (Torino: Edizioni Scientifiche Einaudi, 1959.) 3,000 lire.

IN the autumn of 1959, the B.B.C. Third Programme broadcast a series of three talks on cosmology followed by a discussion at which the rival theories were debated. The short book *Rival Theories of Cosmology* is a direct outcome of these, the content being substantially the same as that of the talks.

Relativistic theories of the universe are discussed by W. Bonnor in the first chapter. This is followed by an account of the steady-state theory, the case being put by H. Bondi, one of the originators of the theory. Finally, R. Lyttleton explains his idea that the expansion of the distant galaxies may be due to the proton having a slightly greater numerical charge than the electron. The volume contains some good illustrations of galaxies, but the book would have been improved by the insertion of a short chapter on some of the observational aspects relevant to cosmology. The text does not presuppose any detailed knowledge of astronomy or cosmology, and within the limitations which are of necessity imposed by this restriction the ideas are put forward very clearly. The book is recommended to anyone who wishes to be introduced to some of the problems of cosmology.

L'Exploration des Galaxies Voisines has been written by an astronomer who has himself worked extensively in the field concerned. After a brief introduction to the nature and classification of galaxies, the problems of estimating extra-galactic distances are considered in some detail. Subsequent chapters deal with the luminosities, diameters, spectra, colours, rotation and masses of galaxies. In addition, there is a chapter devoted to the information about galaxies which has been obtained by means of radio astronomy. The text is well written in a logical sequence; the plentiful illustrations and diagrams are of a high standard. The author intends to write a second volume in which more general topics such as clusters of galaxies will be treated. There is a dearth of up-to-date books about galaxies, and it is to be hoped that the second volume is of the same standard as this first one.

The work by G. Abetti and Margherita Hack discusses planetary nebulæ, diffuse nebulæ and interstellar absorption before describing some of the structural and kinematic properties of the galaxy. The last chapters are concerned with galaxies in general and the rival theories of cosmology. The scope of the book covers a wide range of subjects which are in some cases rather indirectly connected with each other, and the planning of the volume leaves much to be desired. Despite this criticism, some of the individual description is quite good. The book contains some useful material which is not often found in accounts written for the non-specialist.

J. B. Alexander

VERSATILITY OF VACUUM

Progress in Vacuum Science and Technology

Edited by A. S. D. Barrett. Pp. iii + 160. (London and New York: Pergamon Press, 1959.) 70s.

IN recent years vacuum techniques have been introduced into a number of different industrial fields and this book will give the reader a good idea of some of them. The book contains six chapters, each dealing with a particular application and written by an expert in that field. There is also an introductory survey of education in vacuum practice at colleges in the United Kingdom. The contributions have been published as separate articles in the journal *Vacuum*.

Although the book-title includes "Science and Technology", the majority of the chapters are written from a technological rather than a scientific point of view, and are largely descriptive. That on problems in the valve industry does, however, touch on the basic theory more than most. The chapter on the gettering process in receiving valves includes a historical survey of developments leading to the modern type of barium getter. The gas clean-up performance of the latter is given, but the physics of the process is barely mentioned.

The applications of vacuum to metallurgy, fumigation and drying of medical and food products are in complete contrast to valve techniques. These chapters deal with the use of not-so-high vacua on a heavy engineering scale and make interesting and informative reading to those whose knowledge of vacuum is derived mainly from the standard textbooks. The final chapter, on molecular distillation, describes a number of vacuum stills, particularly those of Hickman, who has been largely responsible for recent developments in this field.

A book of this nature is unlikely to be exhaustive, and the most notable omissions are the recent work on ion-pumps and the production and use of ultrahigh vacua, which get only passing mention. The book is well produced and fairly free from misprints. Most chapters have an adequate bibliography; that on molecular stills in particular. Thus this book provides a useful survey of the industrial applications of vacuum, in a form suitable for a busy technologist to read.

R. O. Jenkins

A NEW THERMIONIC GENERATOR

By Prof. D. GABOR, F.R.S.

Department of Electrical Engineering, Imperial College of Science and Technology, London, S.W.7

IT has been known since the investigations of Irving Langmuir in the early 'twenties that the thermionic vacuum diode can be operated as a converter of heat into electric power, but with very poor efficiency owing to the minuteness of the currents. In recent years it was recognized that the efficiency could be raised to an economically interesting level if it were possible to compensate the electronic space charge by a sufficiently cheap supply of ions. Cæsium, with the lowest ionization potential of all the elements (3·9 V.) appeared the most promising, all the more as cæsium ions could be produced by the Langmuir–Kingdon effect, that is to say, by contact with the hot surface of materials with a work function exceeding 3·9 V. In the cæsium diode, as developed by R. W. Pidd *et al.*[1], also by K. G. Hernquist[2] and V. C. Wilson[3], cæsium vapour is ionized by contact with a hot electrode which can be either the emitter itself, or a third, hot electrode. The minimum cost per cæsium ion, taking into account the radiation losses of the hot surface, has been estimated as 100 eV., and as one cæsium ion can compensate the space charge of about 500 electrons, theoretically one ampere could be passed through such a diode at a cost of about 0·2 watt. The experimental figures are appreciably larger.

In the new thermionic device an experimental cost figure of 0·05–0·1 watt/amp. has been achieved, moreover not with cæsium, but with argon (ionization potential 15 7 V.) or with an argon–mercury mixture. Using cæsium, the cost could be reduced by a factor of 3–4, but it is doubtful whether it would be worth while to improve the efficiency further at the expense of the useful life of the device, which is likely to be much shortened by the chemically very active cæsium or other alkali metals.

The principle of the new device is illustrated in Fig. 1. The main discharge occurs between an emitter, which can be heated, for example, by flame gases through a ceramic envelope, and a collector electrode. For reasons to be explained later, it is essential to give the collector a large area relative to the emitter ; it is therefore realized as a cellular structure. The voltage drop in the main discharge space is of the order of a small fraction of a volt only, so that no ionization or excitation occurs in it, and in operation this space appears dark. All ions are supplied to it from an auxiliary discharge between a gauze or similar perforated structure and an auxiliary anode. In operation a small fraction of the electron current shoots out from the dark plasma in the form of fine electron beamlets through the meshes of the gauze, where an acceleration region is formed, with a drop just sufficient for ionization. Owing to the favourable focusing properties of the electric field, a large fraction of the ions thus formed returns through the meshes into the main discharge space. The flow of electrons and ions is illustrated in the left half of Fig. 1, but only schematically, just to illustrate that only a small fraction of the emitter current goes into the auxiliary discharge, while a large fraction of the ions find their way to the cathode. There is some experimental evidence that this is aided by reflexion of the slow ions at the walls of the collector, because increasing the surface of the collector by a cellular structure had a remarkably small influence on the auxiliary discharge required to maintain a certain emitter current. The question mark in the reflected ion path indicates our almost complete ignorance on the subject of the reflexion of very slow ions at surfaces.

It is very essential for the operation of the device that the auxiliary discharge has a positive characteristic, as only this enables thousands of tiny discharges in the meshes to operate in parallel. This is true for all inert gases and also for mixtures of argon and mercury up to a certain proportion of mercury. (For example, 2 mm. argon, with mercury saturated at 80° C.) In pure mercury the elementary discharges tend to have a negative characteristic, and instead of the operation as illustrated one then obtains an arc through a single mesh. In order to make the operation economical the meshes are made sufficiently wide so that the drop in the accelerating region exceeds the ionization potential only a little (80–120 wires per inch). As the anode drop at the auxiliary anode is negative, the total drop in the auxiliary discharge can be appreciably less than the ionization potential, for example, 10 V. in argon, only 7·5 V. in an argon–mercury mixture. The ions are therefore rather slow when they shoot through the gauze, and are slowed down further by collisions with gas molecules, so that they have little more than the gas temperature by the time they reach the cathode.

According to I. Langmuir's theory[4], slow ions coming from a plasma will compensate optimally the space charge of the electrons, emitted by a hot cathode, if the ion current is a fraction $\sqrt{m/M}$ of the electron current, where m is the mass of the electron, M of the ion. This ratio is 1 : 272 for argon, 1 : 494 for cæsium, 1 : 607 for mercury. Though Langmuir's theory was an excellent qualitative guide in the experiments, it might require some quantitative revision because the auxiliary currents in argon were in some experiments as small as 1/100 of the emitter current. Even if all the power in the auxiliary discharge were converted into ionization, this would give only about one ion for 160 electrons, and as the ionizing efficiency was certainly much short of 100 per cent and as some ions must have been lost in the gauze and at the walls, it appears very likely that the ion : electron ratio can be, in fact, better than Langmuir's optimum, again suggesting that there is some reflexion of slow ions.

The picture of the discharge derived from our experiments is illustrated in the right half of Fig. 1. The dark discharge space is filled with a dense plasma of remarkably small electron temperature. At low currents this is only a few hundred degrees in excess of the emitter temperature, indicating that the cathode drop is a few hundredths of a volt only, rising to a few tenths at large currents. As the current density can be of the order of an amp./cm.^{2}, the cathode sheath thickness must be of the order of

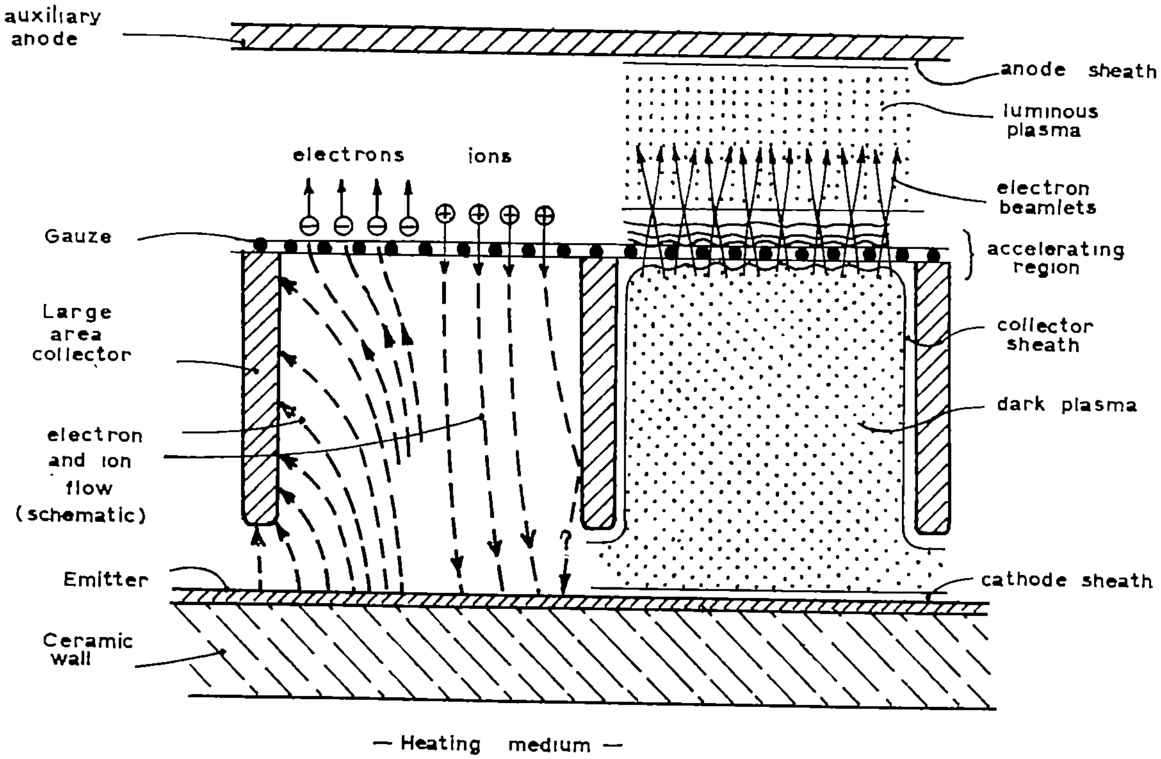

Fig. 1. Schematic section through thermionic generator

1μ. At small currents the plasma has a practically uniform potential; at higher currents a gradient develops. It is a fortunate circumstance that owing to the Ramsauer effect the cross-section of argon to electrons with energies of the order of a few tenths of a volt is very small, but even if it were zero the resistivity of a plasma, as estimated by Gvosdover[5] and by Spitzer[6], is not negligible. At the collector there is a negative drop in all circumstances which we have investigated, and this can be larger than the cathode drop, so that the total voltage drop in the gas space can be negative.

These conditions are further illustrated in Figs. 2 and 3. Fig. 2 is a diagram of the potential from terminal to terminal. As a typical example the emitter work function $\varphi_{\text{em.}}$ may be 2·5 V., the collector work function $\varphi_{\text{coll.}} = 1 \cdot 0$ volt, giving a nominal terminal voltage of 1·5 V. per element. Fig. 3 shows the voltage-current characteristics of the device, theoretically derived but well confirmed by experiments. The theory starts from the assumption that at small currents the discharge space is filled with a uniform, equipotential and, as regards the electron temperature, isothermal plasma. Consider first the case of zero potential difference between emitter and collector. In that case the same random electron current flows from the plasma to equal areas of the emitter or of the collector. The current density at the collector is equal to this random current, while at the emitter it is the difference between the saturated emission and the random current. It follows at once that at $V = 0$ the current is a fraction of the saturated emission current equal to the ratio of the collector area to the total area, that is to say, emitter plus collector. In the diagram it has been assumed that the collector area is three times the cathode area, hence at $V = 0$ the current is 3/4 of the saturation current. The saturation current is limited either by the thermal emission of the cathode, or by the ion supply. By Langmuir's rule the ion-limited current is $\sqrt{M/m}$ times the ion current, and will be proportional to it until thermal saturation is reached. This has been well confirmed by experiments, because it was found that the saturation currents were proportional to the currents in the auxiliary discharge, in any event at low values of the current. With oxide cathodes at high auxiliary currents there is no sharp limiting current because at ion supplies exceeding Langmuir's ratio there will be a non-zero electric field at the emitter, and in composite cathodes field-assisted emission is relatively important.

The same model of a uniform, equipotential and isothermal plasma applied to non-zero values of V gives a current characteristic $I(V)$ in the shape of a hyperbolic tangent function, with a subtangent $4kT/e$, where T is the electron temperature. This was verified, and the value of T agreed well with that obtained from Langmuir probes. At low currents, T was found to be only slightly larger than the emitter temperature. At currents large enough for the plasma resistance to become noticeable this model ceases to apply because the plasma will no longer be equipotential. A good qualitative insight can be obtained by using Steenbeck's principle, according to which the potential profile and the electron temperature will adjust themselves in such a way as to make the total heat dissipation at given current a minimum. The heat dissipation per unit current is $V + 2kT/e$, the second term being the kinetic energy which the electrons give up to the collector, and the electron temperature T must be such as to make this a minimum. As by Spitzer's law the plasma resistance is proportional to $T^{-3/2}$, the electron temperature will increase with rising current, ultimately with the 2/5

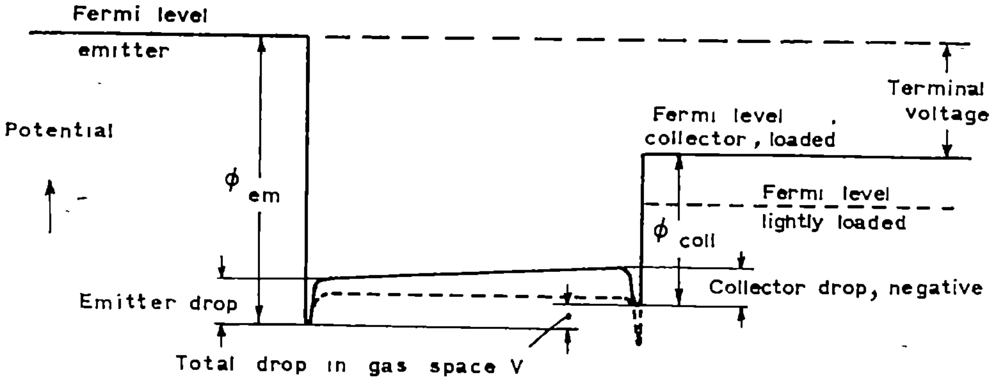
Fermi level
emitter
Potential
ϕ_{em}
Emitter drop
Total drop in gas space V
Terminal voltage
Fermi level collector, loaded
Fermi level lightly loaded
ϕ_{coll}
Collector drop, negative

Fig. 2. Potential diagram. At light loading (broken lines), the plasma has a uniform potential, at heavy loading (continuous lines), the effect of plasma resistance becomes noticeable

power of the current density. This is qualitatively illustrated in Fig. 3, where the base $4kT/e$ broadens with increasing current. Experimentally we have observed an increase of kT/e from 0·11 V. at small currents, to 0·25 V. (2,900° K.) at the largest densities investigated.

This is one of the effects of large currents. The second is a shift in the characteristic, owing to the gradient in the plasma. This, too, is illustrated in Fig. 3. At the point where $I = 0{\cdot}75\ I_{max.}$, which would correspond to $V = 0$ if the plasma were equipotential, the characteristic at higher current densities is shifted to the right by an amount V, which is the drop in the gas space. To obtain an idea of this drop note that Spitzer's law gives a plasma resistivity of the order:

$$\rho = 1{\cdot}0\ (T/1{,}000)^{-3/2} \text{ ohm. cm.}$$

At the low electron temperatures with which the thermionic converter operates this is quite appreciable. Even at $T = 2{,}900°$ K. it is still 0·2 ohm. cm. Thus, if we had a plane emitter faced by a plane collector at a distance of 1 cm., we should have at 4 amp./cm.^{2} a drop of 0·8 V., which is far too large

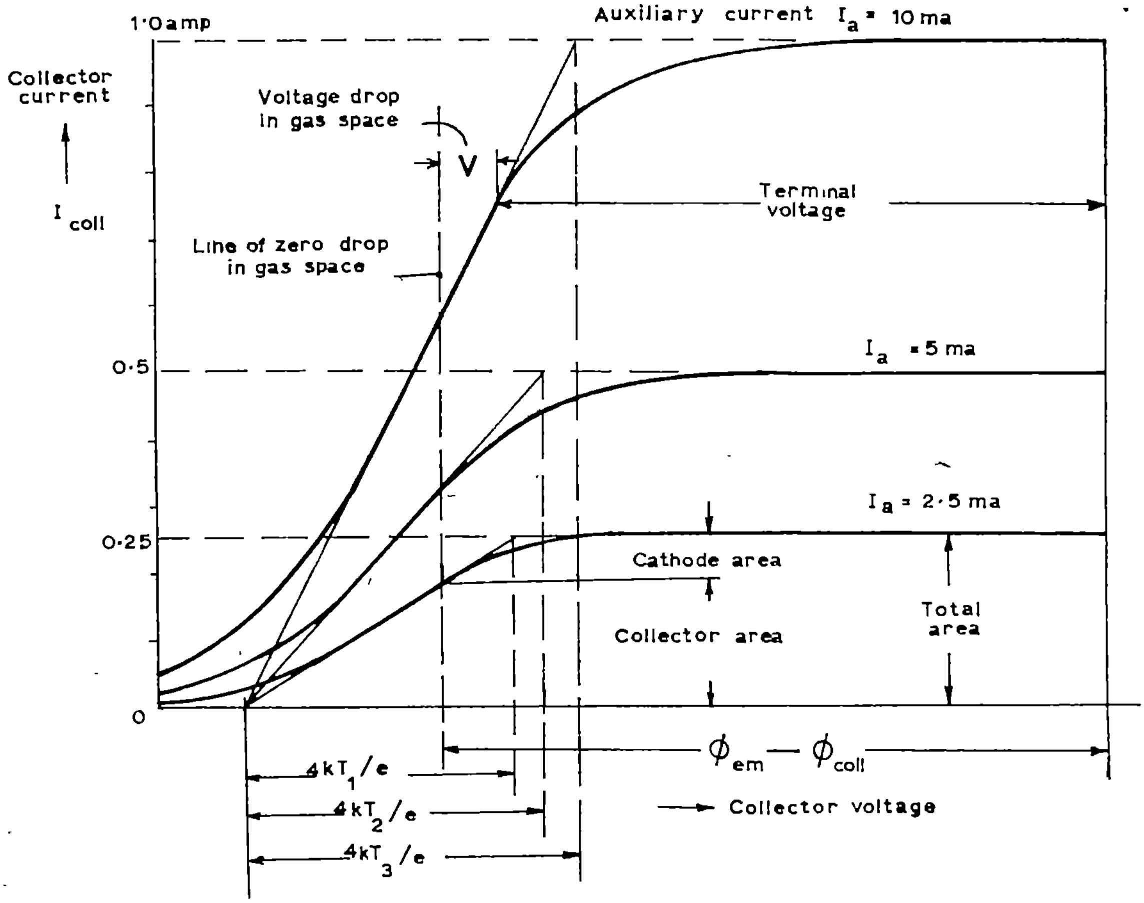

Fig. 3. Current-voltage characteristics, schematic

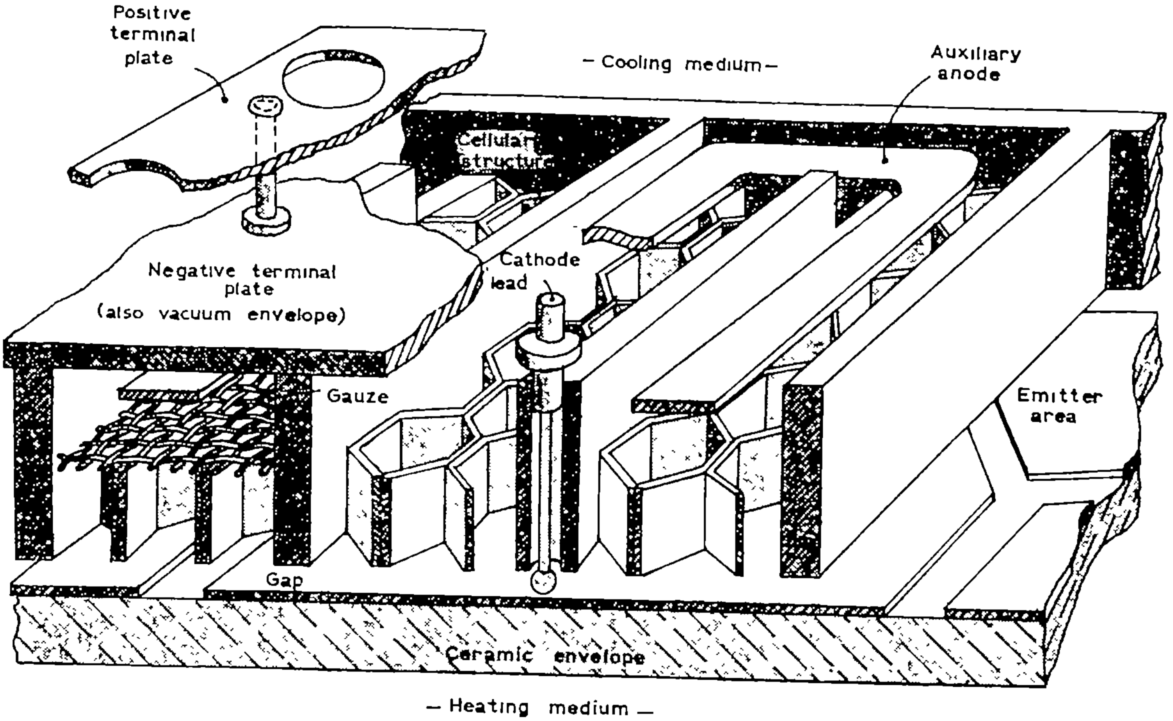

Fig 4. Thermionic generator element

to be tolerable. This has an important consequence for an economic design. It was shown previously that in order to reduce the voltage drop, the collector area must be large relative to the cathode area. It is seen now that in addition we must also reduce the absolute dimensions of the emitter-collector assembly.

Fig. 4 shows a design of a thermoelectric generator in which this is taken into account. The collector consists of a cellular metal structure, of a material with good heat conductance, interspersed with bars of sufficient cross-sections to conduct the heat without a large temperature-drop to a terminal plate, which is immersed in the coolant. The cells have small dimensions, say 3–5 mm., and they are within a small distance, say 1 mm. from the face of the emitter, so that the plasma resistance is that of a pair of parallel plates at about 2 mm. distance. This is sufficient for current densities of the order of 4 amp./cm.^{2}; for higher densities the dimensions ought to be further reduced. It is seen that the fineness of this 'microstructure' does not prevent the realization of large units.

A metal gauze is applied to the top of the cellular structure, separating the auxiliary discharge space from the main space. This second space contains a meandering auxiliary anode. It has been found that a single auxiliary discharge space is preferable to one broken up into smaller units, as it ensures that the discharge will spread over the whole surface of the gauze.

While most prime movers improve in efficiency when they are scaled up, large thermionic generators beyond a certain size suffer from a grave disadvantage. This is the self-magnetic field of the generated current, which tends to prevent the emission by returning the electrons to the emitter. One can reduce this somewhat by admitting the currents not at one point but evenly over the periphery of the device, but only up to a certain limit. As an example, let 25 gauss be the limit of the magnetic field parallel to the emitter surface which one can tolerate. Such a field is produced by 20 amp./cm. With a current density of 4 amp./cm.^{2} this limit is reached in a square of 20 × 20 cm., at a current of 1,600 amp. If one makes the unit larger the current will not increase, because the emission will be cut off beyond the critical square. Larger units must consist 'magnetically' of sub-critical units. One way of achieving this is shown in Fig. 4. The emitter area is divided into hexagonal sub-areas, each with its own cathode lead in the centre. If these are small enough the loss by magnetic fields can be made negligible. For example, if each sub-unit supplies 100 amp. and has an area of 25 cm.^{2}, the critical area around the lead in which the magnetic field is more than 25 gauss has a radius of 0·8 cm. and an area of 2 cm.^{2} only, that is, 92 per cent of the emitter area is utilized. An advantage of this design is that each cathode lead acts also as a mechanical support for the ceramic envelope against the atmospheric pressure. Moreover, one finds that if these leads are made, for example, of tungsten or molybdenum, of such thickness as to present the optimum compromise between ohmic drop and heat conduction, they will be flexible enough to follow the differential thermal expansion between the emitter and the collector structure. All cathode leads are connected with a positive terminal plate which is perforated and immersed in the coolant. Such units can be made in any size, and they need not be necessarily plane.

The efficiency of thermionic generators has been calculated by several authors, in particular by J. M. Houston[7]. It is

$$\eta = \frac{\varphi_{\text{em}} - \varphi_{\text{coll.}} - RI - (\text{drop in discharge}) - (\text{cost of ions in W./amp.})}{\varphi_{\text{em.}} + 2(k/e)T_{\text{em.}} + (\text{heat conduction/amp.}) + (\text{radiation/amp.})}$$

RI is the internal ohmic drop. The optimum compromise between this and heat conduction is usually at RI = 0·05–0·1 V. The conditions which favour a small voltage drop in the discharge and reduce it to 0·1–0·2 V. or less have been already discussed, and it has been mentioned at the beginning that the cost of ions can be made as low as 0·05–0·1 V. in the present device. The most important parameters for good efficiency are therefore the work functions $\varphi_{em.}$ and φ_{coll} and the radiation per ampere emission. The heat conduction through argon at about 2 mm. pressure plays a small part in the losses. The work function of the collector must be evidently made as small as possible. It can be reasonably expected that it will be possible to reduce it to 1 V. This leaves us with finding a suitable compromise between the emitter work function and the radiation loss per amp. current. The best compromise depends on the emitter temperature. If this is given, one has the choice between a larger $\varphi_{em.}$ giving a higher terminal voltage but smaller currents and thereby larger radiation loss per ampere, or a smaller $\varphi_{em.}$ with smaller radiation losses. At emitter temperatures of 1,500°–1,800° C. the choice is largely in the region $\varphi_{em.}$ = 2–3 V., and working out examples with existing emitter materials one finds that the advantage is on the side of the lower work functions. These give not only better efficiencies but also higher power densities, that is, better utilization of space. With present cathode materials one can contemplate efficiencies of the order of 20–30 per cent and power densities of the order of 4–6 kW./sq. ft.

It is a unique advantage of the new device that it allows the direct generation of alternating current. The device becomes at once non-conducting if the auxiliary discharge is extinguished, and can be made conducting in a fraction of a millisecond by re-striking it. Thus, if one takes two of these devices and connects them in push–pull over a transformer as output, so that they will send currents into the transformer alternatingly, one obtains alternating power. One can supply this to an a.c. network by deriving the small auxiliary power from the mains in synchronism with it. The efficiency in a.c. operation is somewhat smaller than with d.c. because heat conduction and radiation go on also during the non-operative half-cycle, but the reduction in efficiency (say from 30 to 22 per cent) might well be outweighed by the gain in the cost of the plant.

Though the investigations of the gas discharge in the new thermionic device are not yet closed, these phenomena are now sufficiently understood to form the basis for the design of practical generators—given suitable materials. Further research must be directed towards the three problems in material technology presented by this or any other thermionic converter. The first is a cathode material with 2–2·5-V. work function, with small radiation emissivity, capable of supplying 4 amp./cm.[2] or more at temperatures in the region of 1,200°–1,800° C. The second is a suitable collector with high reflectivity and low work function. In a self-contained generator in which the coolant temperature is, say, 100° C., this ought to be about 1 V. In a generator used as a 'topper' for a steam turbine, with the coolant at 5–600° C., it can be 1·5–1·8 V., and there exists a fairly wide range of suitable materials. Such a converter would amount to a boiler which turns a part of the fuel energy directly into electric power, the rest into steam.

The third problem, probably the most difficult, is that of a ceramic envelope impervious at 14–1,800° C., capable of operating for long times when exposed to flame gases and with a high thermal shock resistance. This last problem would not arise in one line of possible applications, namely, as a converter in an atomic reactor in which the nuclear fuel could be included in the envelope. This application, however, will not mature until it is proved that fuel elements can operate at such high temperature, and do not contaminate the emitter, the collector and the gas with fission products. A small amount of gas contamination, as well as some measure of gas transmission in the ceramic envelope, can be dealt with by titanium pumps or the like, which eliminate chemically active contaminants, but not argon or other inert gases.

Provided that these problems can be solved satisfactorily, combining a low cost with a long life, there is a wide field of potential applications for thermionic generators, in supplying d.c. power for electrochemical plants, for traction, for small self-contained generator units, and for improving the efficiency of large steam or nuclear power plants.

My thanks are due to Mr. H. A. Fatmi, who has carried out the experimental work, and to the National Research Development Corporation, which provided the financial support.

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THE MARITIME UPLANDS OF BRITAIN: INVENTORY AND PROSPECT

By JAMES A. TAYLOR

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THE alleged equability of British climate is a notion derived not only from a traditional consideration of exclusively mean meteorological data but also from the fact that the vast majority of the major synoptic meteorological stations of Britain are situated at low elevations (see Fig. 1). It is well known that weather, climate and ecological potential deteriorate with increasing altitude in the British Isles, but the remarkably rapid rate of deterioration, even for a maritime climate, is much less publicized. If, for idealized maritime and continental sites (Fig. 2), horizontal lines A (low-level) and B (high-level) for the former, and lines A' (comparable low-level) and B' (comparable high-level) represent growth thresholds (for example, 42° F.) crossed upwards in spring and downwards in autumn

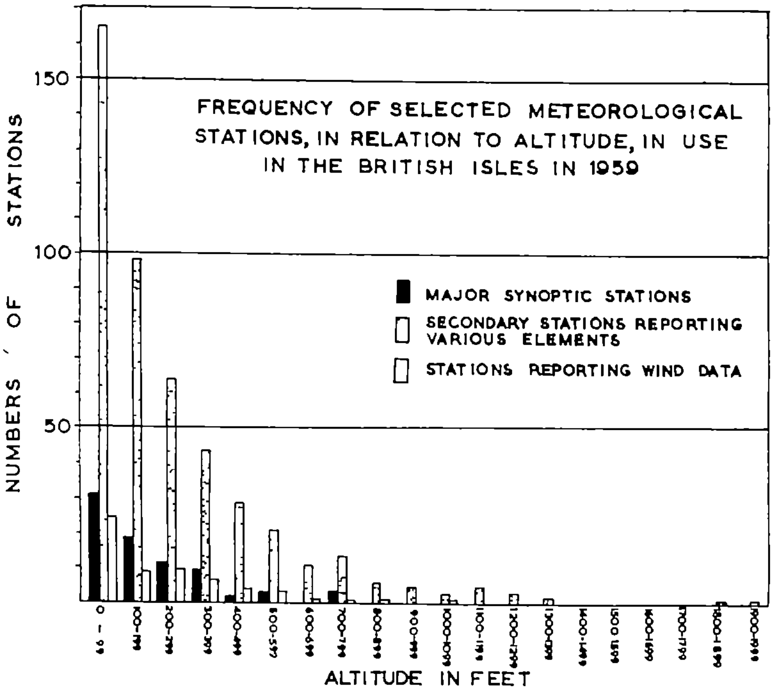

Fig. 1

by the mean monthly temperature curves, then the vertically shaded and stippled areas represent the relative amounts of growth potential at each site and elevation. Clearly, the maritime site, despite its earlier and longer season, has a lower growth potential, which is reduced rapidly with elevation. The continental site, however, despite its later and shorter season, has a greater growth potential which is reduced much less rapidly with elevation. It follows that agricultural limits, tree-lines, snow-lines, etc., occur at much lower elevations at maritime, compared with continental, sites.

This sharp ecological gradient[1] is the expression of decreasing temperatures, especially maximum temperatures, and of increasing rainfall, derived more from the elevation and modification of frontal structures than the simple rise of moist air streams. Increasing cloud cover and persistence, relatively low-cloud bases, high humidities and the liability to mist or fog accompany the increasing rainfall. Complementarily, evaporation (when it is possible) and sunshine amounts are reduced to a minimum. Frost incidence, snow-fall, snow-cover[2] and wind-speed[3], with few exceptions, also increase with altitude. All these trends imply a progressive, exceptional rate of deterioration of meteorological and ecological conditions with altitude in Britain.

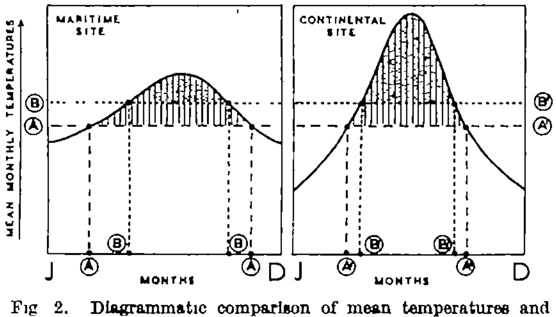

Fig 2. Diagrammatic comparison of mean temperatures and growing season at maritime and continental sites

The techniques of pollen analysis and carbon-14 dating have enabled a reconstruction of the vegetation chronology since the end of the last major glaciation (about 12000 B.C.). Tundra was eventually succeeded first by birch and pine forests and then by mixed, deciduous oak woodland which, by the post-glacial climatic optimum (6000–2000 B.C.), attained elevations substantially higher than the 2,000-ft. contour. During the early Mesolithic (8000–6000 B.C.), upland Britain was extensively covered with birches and pines under a relatively dry, continental 'Boreal' climate. From the late Mesolithic (5000–2800 B.C.) through to the Neolithic and the end of the Bronze Age (about 500 B.C.), thick, deciduous woodland containing much oak and alder dominated at first under a warmer, oceanic, 'Atlantic' climate from about 5000 B.C. to about 3000 B.C., and later under the drier régime of the 'Sub-Boreal' phase from about 3000 B.C. to about 500 B.C. Afterwards, the 'Sub-Atlantic' phase (about 500 B.C. *et seq.*) witnessed a resumption of a moister climate and progressively slow, then rapid, deforestation of upland Britain by man, and the creation of the moorland landscapes we know to-day.

Some of the more striking features of the 8,000 years of the post-glacial period are embodied in contemporary soil profiles in upland Britain. The deciduous forest climax was longer and more potent than the preceding birch and pine forests, and promoted the development of deep brown earths. The qualities of colour and texture in the *B* horizons of many contemporary hill soils at present under an incongruous grass vegetation may be attributed to this period. The thick mantle of hill peat developed on typically flattish or gently sloping sites accumulated in particular during the oceanic 'Atlantic' phases. On the other hand, the contemporary semi-natural moorland grasses have formed dense, matted root layers, forming the familiar 'organic-top' of many hill soils, below which a bleached A_1 horizon, greyish in colour, is characteristic. The local development of iron pans or organic pans at the top of the *B* horizons may create impedance within the profile, as evidenced in the mottling of the lower section of the A_1 horizon. These features are expressed in the peaty-gley-podzol which is the most common soil in upland Britain. They are the creation of past vegetations and climates as well as contemporary vegetation and climate, and of past, as well as present, usages by man and his grazing animals.

It is surprising that man's continuous, if extensive, use of the hills in Britain has not facilitated more intensive inquiry into the hill environment itself. The upland soils have been least modified by agriculture and could provide more direct evidence of post-glacial pedogenic processes than their intensively cultivated lowland counterparts. Yet the latter take precedence over the former in the programme of the Soil Survey. Again, the semi-natural upland vegetation is regarded as something to be improved and 'forced' for grazing purposes—rather something which, in fact, has been degraded by grazing. Detailed vegetation maps of the uplands of Britain scarcely exist. The upland climates of Britain, except for individual short-period investigations, remain taken for granted and often incompletely appreciated. The relatively low contemporary economic value of the uplands of Britain has been a major factor in this neglect. Once areas of pioneer colonization by primitive man, these uplands have now become depopulated and devalued by modern man. The agricultural lowlands and the coalfields have claimed priority in geological and pedological surveys[4]. The popular comment, "all our vegetation has gone" has perpetuated the almost peculiarly British notion that we live in a conquered environment virtually emancipated from the extremes of Nature. In the hill areas nothing could be further from the truth. Economists to-day repeatedly insist that the hills should be left to water conservation, afforestation, national parks and nature reserves. Hill soils and hill climates have been ecological proof of this doctrine for thousands of years. However, the relegation of sheep-farming and promotion of water conservation and afforestation requires careful and comprehensive planning, which should consider ecological, pedological and sociological argument, in addition to the purely economic considerations.

Cattle depopulation and the gradual disappearance of older sheep from upland Britain have caused a decline in intensity of grazing in recent decades. Consequent regeneration, especially of bracken, has proceeded at locally alarming rates. Bracken is apt to colonize the deeper, non-impeded brown earths which are normally the most productive and versatile soils on the slopes ; areal loss is thus coupled with loss of good land. Much Welsh *ffriddoedd* has been transformed into bracken fields dotted extensively with scrub oak, hawthorn and dwarf gorse. The monopolistic, selective grazing by sheep has allowed species such as *Nardus* and *Vaccinium* to dominate many associations at one time richer, for example, in *Molinia* and *Agrostis*. Heather monocultures (for example, in parts of the Scottish Highlands and on the Llandegla Moors in north-east Wales) induce relatively strong soil-podzolization and confine land use to extensive sheep grazing, usually by Scottish blackface.

The dairying revolution of the period *circa* 1933–52 is still registering in remote parts of the uplands of Britain, affording modern evidence of the traditional delay in cultural response which has characterized the Highland Zone of Britain since the earliest times. Some mid-altitude farms have recently introduced milk production, provided they were located within, or within reach of, the catchment of a milk lorry. Many what may be termed 'natural rearing farms' have been modified in this fashion in response to the temporary and, in many ways, excessive economic provocation embodied in the attractive marketing and price facilities and the regular income offered by the Milk Marketing Board. At the same time, sheep farmers face the annual problem of the high cost of wintering their flocks on lowland farms. The comparatively short-term economic and political arguments which initiate these trends and problems should always be qualified by the excessively sharp decrease in flexibility and adaptability of farming systems, and of farms with large proportion of 'hill', with increasing altitude in the maritime uplands. Also, the law of systematic and regular integration between hill farm and lowland farm is herein revealed and is inescapable.

Relatively recent allocations of hill land have been made to the Forestry Commission, to water and electricity authorities, to the National Parks Commission, the National Trust and the Nature Conservancy, to the Army and to the British Atomic Energy Authority.

To what extent the introduction of conifers (for example, since 1927 in Dovey Forest) on partially podzolized soils has induced further podzolization is debatable. Preliminary evidence[5] indicates that little, if any, perceptible degradation has taken place, in particular in Wales, where parent materials in general do not possess an adequate combination of extreme porosity and acidity, as for example occurs in the Barton Sands of the New Forest, where a period of only twenty-five years under conifers has been enough to create advanced podzolization[6]. Afforestation, on the other hand, has helped to check rural depopulation and has provided good, new access roads for many hill farms.

The economic priority claimed by water and electricity authorities in the creation of reservoirs and impounding of water must be assessed against what often amounts to the effective elimination of the antecedent forms of land usage. Planted trees may share the slope with sheep, trees and sheep may occupy land within a national park, but a flooded valley must lose not only its trees and its sheep but also its land as a whole, good, bad or indifferent, and its settlements, people and history. The valley, as it were, dies. But a new lake, a new landscape is created, and miles away a new industry expands and flourishes.

Extensive sections of hill country in Britain have been declared as national parks and provide extended scope for tourism, the industry which to-day can claim one of the largest individual incomes in the country. Tourism, together with water storage, represents potentially one of the most profitable uses which can be made of the uplands of Britain, provided modernized accommodation and hostelries of the right calibre can attract an adequate range of tourists. This will require continual improvements in communications, especially by road.

More limited hill areas have been taken by the Nature Conservancy and the National Trust, and afford opportunities for studying the relation of plant regeneration to soil, slope and climate. Evidence derived from such an investigation will confirm the ecological law that, with increasing altitude in Britain, it becomes progressively—and soon remarkably—less profitable to create artificial soil conditions to introduce lowland species of plants or plants bred specifically to cope with more rigorous environments. Whatever success is achieved on a re-seeded hill, grazing is notoriously short-lived and uneconomical.

The availability of large areas of unpopulated hill has among other factors favoured the creation of army training camps and, very recently (1960–61), the establishment of nuclear power stations, such as that at Trawsfynydd in Merionethshire.

Hill farming leans on Government subsidies. Forestry represents a long-term Government investment. Forestry, in the long run, is relatively more profitable per acre and employs more men per acre[7] than hill-farming which, for the most part, retains the better land on the lower slopes and plateaux and valley bottoms, permitting plantation on the higher, or more exposed, or more steeply sloping, ground. It is reasonable to argue that, given equal status in land allocation, forestry would be even more profitable than hill-farming and on lower ground even more profitable still. But sheep farms are indigenous, as it were, to the hills; the plantations are intrusive. Forestry can exploit the more remote hill land more effectively, but at the cost of a longer journey-to-work. The sheep farm (except for certain types of fragmented holding), is a more self-contained labour unit with traditional, external contacts for marketing and social excursions. Rearrangement and amalgamation of holdings might be advocated to promote, rather than reduce, these contacts. New villages created *ab initio* by the Forestry Commission pass through a very difficult formative period until the second generation, born in the village, becomes adult. It is wise to extend old villages where a traditional community spirit already exists. Under-grazing by sheep has allowed extensive regeneration and loss of hill grazings, and soils undisturbed under matted grass (and derived organic top-layers) for many years become badly aerated and deficient in moisture in dry spells. Re-introduction of store cattle on the hills to graze with the sheep would help to reduce degeneration of grazings. In contrast, preliminary treatments for tree-planting often include deep ploughing, especially when drainage is poor and, although catchments may become temporarily flooded by the improved drainage, the mechanical disturbance and overturning of the soil breaks up the root mat, improves aeration and brings relatively richer *B*-horizon material to the surface. On steep slopes, however, there is a danger that erosion might occur.

The implication of the foregoing paragraphs is that whereas forestry must in Britain claim economic priority over hill-farming virtually to the extent of being permitted access to some of the better land, the deep-rooted traditions of the countryside and its settlement pattern remain centred on its sheep farms and associated hamlets. Also, the implication is that the two can be usefully integrated rather than being allowed to compete; this point will be developed later.

Clearly, the future expansion of industry in Britain and the maintenance of a national economy based on adequate exports will demand more extensive and more efficient harnessing of the water resources of the country, in particular in the Highland zone. Thus, water storage both for industry and for power development must claim the highest priority in the planning of upland land use. On the whole, the afforestation of catchments of reservoirs might be favoured, causing further shrinkage of areas devoted to hill-farming. Sheep are tolerated more on the catchments than cattle as a rule; thus the mixed grazing advocated above on ecological grounds is not always practicable. The relatively small areas taken by the Nature Conservancy, the National Trust, the Army and the Atomic Energy Authority are fully justified in terms of urgent national requirements, and the functions of those organizations. The larger national parks are equally justified on grounds of amenity and tourism. It should be asserted, however, that sites of supreme ecological interest or natural beauty should, so far as possible, not be adopted either for military training or for the establishment of nuclear power stations.

In summary, the general order of priority of land use in the uplands of Britain, according to contemporary economic trends, would be as follows: (1) water conservation for industry and for power; (2) afforestation, including the establishment of shelter belts; (3) stock-farming, including store cattle as well as sheep on reorganized amalgamated holdings.

Within this broad framework of priorities, the local priorities concerning (*a*) tourism, both coastal and rural, winter and summer; (*b*) nuclear power stations; (*c*) military and conservation allocations seem justifiable, provided ecological and æsthetic arguments are satisfied.

The creation of a reservoir eliminates all other forms of land use from the site. Cattle-grazing is not encouraged on the catchment of the reservoir, where afforestation is favoured rather than sheep grazing. Forestry, claiming slight relative economic priority, may in the future deserve better hill land at the expense of the adjacent sheep farms, and even some lower ground at the expense of lowland farms. Afforestation blocks may reduce the internal mobility of a sheep farm. The forests may have been sited long distances from existing settlements, where there is competition for a limited and ageing labour force. New settlements vie with old settlements for people and social and religious privileges. External (for example, non-Welsh) forces in the form of new jobs and new workers compete with local tendencies to conservatism and conventionality. How can a declining population maintain cultural integrity and assimilate incoming cultural influences without suffering dilution? How can old and new elements in the labour force continue to tolerate life in remote hill areas, when for many individuals it probably hangs at this moment by the slender penetrating thread of the television screen? How long will it take for the influence of the progressive emancipation of generations of women and mothers to encompass all rural areas? How long will parents and local authorities tolerate an educational system which permits variety in local opportunity for children at the age of 11+ years and demands the steady annihilation of the country schools within the devouring, centralizing orbit of large, new, cumbersome secondary schools to which rural children are 'exported' and 'imported' daily over many miles? How can this picture of all-round competition and collective decline be reconciled?

Reconciliation must lie in integration, which necessitates some degree of planning. The location of water storage schemes should be arranged so as to create a minimum of dislocation of existing settlement and economy. The Forestry Commission should be encouraged to continue to plant above the commercial tree-line for shelter purposes on sheep farms and allowed to experiment on lower ground to ascertain possible increases in yield. Sheep farmers should be educated to accept some degree of reorganization and amalgamation of holdings as the price to pay for greater economic and social security. The re-introduction of store cattle would give more flexibility to the farming system. The occupiers of the smaller holdings should be encouraged to take part-time jobs in the Forestry Commission which should be made available in its programmes of future expansion. For time immemorial the earlier inhabitants of the hills combined several tasks, for example, domestic craft, mining, sheep farming, cattle farming, and the basis of their success was co-operation, especially during the vital summer months when the mountain pastures were systematically grazed. These ancient laws of job combination and co-operation probably still apply even in the different economic world of to-day. New settlements should be representative of all the labour force and not confined to one type of worker, for example, forestry employees. Moreover, they should be attached to, or be within easy reach of, existing settlements and amenities. Improvement of communications, achieved by the Forestry Commission, for example, should go hand in hand with other forms of planning land use, making the economy more efficient and attracting a bigger and more versatile tourist trade.

These anticipated plans imply change in one form or another. The view that rural cultures (for example, Welsh culture) should not tolerate contamination should be weighed against the inevitability of the economic decay that must accompany their continued isolation.

This commentary owes much to a symposium on "Hill Climates and Land Usage, with special reference to the Highland Zone of Britain", convened by the author in Aberystwyth in March 1960. A memorandum including ten papers was compiled, based on the discussion, and circulated as *Mem.* No. 3, Aberystwyth. Grateful acknowledgment is also made to Prof. Gordon Manley for many valuable criticisms.

[1] Manley, G., *Geog. Rev.*, **35**, 408 (1945).
[2] Manley, G., *Geog. J.*, **103**, 241 (1944).
[3] Gloyne, R. W., in *Hill Climates and Land Usage with Special Reference to Highland Britain*, edit. Taylor, J. A., *Mem. No.* 3, Aberystwyth, 23 (1960).
[4] Taylor, J. A., *Geography*, **145**, 65 (1960).
[5] Taylor, J. A., *Hill Climates and Land Usage, etc.*, *Mem. No.* 3, Aberystwyth, 65 (1960).
[6] Davies, R. I., Department of Agricultural Chemistry, University College, Bangor (private communication).
[7] Report by the Natural Resources Technical Committee. Forestry, Agriculture and Marginal Land (H.M. Stationery Office, 1957).

NEWS and VIEWS

The Royal Society of Edinburgh : Awards

THE following awards have been made: *Makdougall–Brisbane Prize* (1958–60), to Mr. Albert G. Long, Duns, Berwickshire, for several papers on palæobotany published in the *Transactions* of the Society during the period of the award. *Dr. W. S. Bruce Memorial Prize* (1960), to Mr. J. MacDowall, Meteorological Office, London, for his work in the fields of Antarctic meteorology and geomagnetism and as leader of the Royal Society of London Expedition to Halley Bay, 1958 (this Award is made jointly with the Royal Physical Society and the Royal Scottish Geographical Society). *Bruce–Preller Lecture* (1961): Dr. V. A. Eyles, formerly H.M. Geological Survey, London and Edinburgh, has been invited to deliver the Bruce–Preller Lecture, 1961, at the ordinary meeting of the Society on June 5.

New Fellows

THE following have been elected Fellows of the Royal Society of Edinburgh: Dr. F. Alexander, reader in veterinary pharmacology, University of Edinburgh; M. Campbell, secretary, Department of Agriculture and Fisheries for Scotland; Dr. A. J. O. Cruickshank, reader in electrical engineering, University of St. Andrews; Prof. R. B. Dingle, professor of theoretical physics, St. Salvator's College, University of St. Andrews; Dr. P. S. Farago, senior lecturer in natural philosophy, University of Edinburgh; Dr. P. B. Fellgett, principal scientific officer, Royal Observatory, Edinburgh; K. B. Fraser, senior lecturer in botany, University of Aberdeen; Prof. A. Haddow, professor of experimental pathology in the University of London, and director of the Chester Beatty Research Institute, London; W. T. Harry, lecturer in geology, University of St. Andrews; Prof. A. W. Hendry, professor of building science, University of Liverpool; Prof. J. S. S. Inglis, professor of animal husbandry and preventive medicine, University of Glasgow; Dr. H. Lister, lecturer in polar studies, University of Durham, Department of Geography, King's College, Newcastle upon Tyne; D. Lowe, horticulturist, Elvingston, Gladsmuir, East Lothian; Dr. R. C. Mackenzie, head of the Department of Pedology, Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen; B. Noble, senior lecturer in mathematics, Royal College of Science and Technology, Glasgow; B. B. Parrish, Department of Agriculture and Fisheries for Scotland, Marine Laboratory, Aberdeen; Prof. P. L. Pauson, Freeland professor of chemistry, Royal College of Science and Technology, Glasgow; Dr. R. J. T. Pennington, research fellow to the Neurological Unit, Medical School, King's College, Newcastle upon Tyne; Dr. B. B. Rae, assistant to the Director of Fisheries Research, Scotland, Department of Agriculture and Fisheries for Scotland, Marine Laboratory, Aberdeen; Prof. J. R. Raeburn, Strathcona-Fordyce professor of agriculture in the University of Aberdeen; R. M. Sillitto, lecturer in natural philosophy, University of Edinburgh; Dr. F. Smithies, lecturer in mathematics, University of Cambridge; Dr. D. W. N. Stibbs, Napier professor of astronomy in the University of St. Andrews, and director of the Observatory; Dr. A. B. Taylor, Registrar General for Scotland.

Physics at Manchester : Prof. E. B. Paul

DR. E. B. PAUL, who has been appointed to a chair in the Department of Physics of the University of Manchester, was educated at Mallorytown and Brockville Schools and at Queen's University, Kingston, Ontario. From 1942 until 1946 he held an appointment with the National Research Council of Canada at Ottawa and took part in the work on nuclear reactions sponsored by that body in connexion with the development of the Chalk River Laboratories. During 1946–48 Paul studied at Cambridge, where he worked with the 1-MV. high-tension generator of the Cavendish Laboratory and took his Ph.D. degree. He then returned to the Physics Division of the Chalk River Laboratories and undertook an extensive investigation of neutron activation cross-sections which has been widely quoted. Following this, he turned his attention to proton-induced reactions and was for the next few years a leading member of a group which has published many papers on resonant processes in the light nuclei. The high standard of this work both in experimental technique and in theoretical analysis has contributed greatly to the international reputation of the Chalk River Laboratories. In 1955 Paul was appointed to the Atomic Energy Research Establishment, Harwell, where he is now in charge of nuclear research using electrostatic generators, and is deputy head of the Nuclear Physics Division. Paul is well known for his interest in nuclear structure; he was the first to point out that the collective model could give an excellent account of the properties of levels of light nuclei. His ability in interpretation and his wide experience of high-tension work will be of great service to Manchester, where a vigorous programme of research in low-energy nuclear physics is already in being.

Prof. H. E. Hall

DR. H. E. HALL, who has also been appointed to a chair of physics at Manchester, is only thirty-two. He was educated at Latymer Upper School and Emmanuel College, Cambridge. He went to the Royal Society Mond Laboratory in 1952 and quickly made his mark as an exceptionally skilled experimenter who was equally at home in the construction of delicate equipment and in the mathematical analysis of his results. He carried out a variety of researches on liquid helium which have led to a significant elucidation of our understanding of its properties. Probably the most important contribution to this understanding was an elegant experiment, in collaboration with Dr. W. F. Vinen, in which they showed that second sound was attenuated when propagated in rotating liquid helium II; this demonstrated very directly the existence of vortex lines. In 1958 he was appointed lecturer in the University of Manchester, and has contributed to the development there of a vigorous low-temperature group; he has also expanded his interests and taken up some interesting problems concerned with the Mössbauer effect.

British Scientific Attaché in Paris: Mr. R. V. Melville

MR. RICHARD MELVILLE, of the Department of Scientific and Industrial Research, has been appointed scientific attaché to the British Embassy in Paris. He will advise the British Ambassador on scientific matters and report on French scientific and technical development in the civil field. Mr. Melville, who is forty-seven, will hold the rank of senior principal scientific officer. He has been with the Department of Scientific and Industrial Research since joining the Geological Survey and Museum in 1938. He has recently been on secondment to the International Commission on Zoological Nomenclature as assistant secretary, which has brought him into close contact with French scientists. Mr. Melville, who is French-speaking, has also taken part in scientific expeditions in France and French North Africa. Educated at Wellington College and in the United States, he graduated at the University of Reading in 1936, obtained a special honours degree in geology the following year and obtained his M.Sc. in 1941.

Prof. Peter Pringsheim

PROF. PETER PRINGSHEIM, who will be eighty years of age on March 19, was born in Munich, son of a distinguished mathematician, his mother being formerly an actress. He grew up in a home of culture, music and of literary interest. His sister married Thomas Mann. Prof. Pringsheim spent some time in Cambridge with J. J. Thomson, but then joined Rubens in 1909. His career was profoundly affected by the two World Wars. In 1914 he was attending the British Association meeting in Australia when war was declared and he was interned for its duration. During his enforced leisure he wrote the text of his monograph on fluorescence and phosphorescence which, together with his work in the field of fluorescence of vapours, established him as an authority. Soon after his return from internment he was appointed professor in the University of Berlin, where he remained until dismissed in 1933 because of his partly Jewish descent. He moved to Brussels with his Belgian-born wife; but in 1940 was overtaken by another World War, being arrested and taken to a camp in France. Fortunately, his American friends secured his release and he was able to reach the United States to work first in California and later in the Argonne National Laboratory, although he was not reunited with his wife until 1946. In 1954 he retired to Antwerp but maintained his interest in luminescence.

In the early 'twenties Prof. Pringsheim extended his interest in luminescence to the fluorescence of organic molecules in condensed systems. He contributed to the *Handbuch der Physik* in his field. The years of the Second World War led to a revision of his book, and in 1946 a new, monumental text was ready, perhaps the last possible attempt anyone could make to provide a comprehensive review of luminescence in the gaseous, liquid and solid states. Although he regretted that so much of the war-time advances in luminescence was released after the publication of his book, Pringsheim's text still stands as a landmark in luminescence, with its bibliography only a little short of two thousand references. He completed his own research interests by a post-war incursion into the problems of luminescence and colour centres in the alkali halides, reviving a previous interest in the fluorescence of heavy metal ions complexes in solution. He is one of the few people who has attended almost all the major conferences in his subject and it was a pleasure to see him, even in retirement, participating in the Paris Conference of 1956.

Effects of Radioactivity in the Sea

RESEARCH on the effects of radioactivity in the sea is to be undertaken by the International Atomic Energy Agency under an agreement recently concluded between the Agency, the Government of Monaco and the Oceanographic Institute in Monaco. The research, which will be conducted at the Scientific Centre of the Government of Monaco, is related to the problem of possible pollution of the sea through the deposition of radioactive materials. It is aimed at studying: (*a*) the movement of water and marine organisms and the deposition of organic and inorganic matter; (*b*) the distribution in marine organisms of radioactive materials already existing or that may be introduced into various locations; (*c*) the effects on marine life of radioactive materials at various concentration-levels. The programme has been approved by the Agency's Scientific Advisory Committee, which is composed of seven leading scientists from different countries. The research, which will start soon, is planned to extend for three years. The laboratory and working facilities of the Scientific Centre in the Oceanographic Museum and a wide variety of electronic and monitoring equipment will be made available by Monaco. The Oceanographic Institute will put at the disposal of the project a number of valuable facilities, including marine biology laboratories, oceanographic vessels and some specialized fishing equipment. Certain other facilities, including the 360-ton oceanographic ship *Calypso*, may also be available through the Institute. The Monaco Government will contribute 200,000 French new francs for each year of the research project. The Agency will appoint a chief scientist to be in charge of the research and provide other personnel as well as specialized equipment and technical supplies. Agency fellowships will be awarded for training in connexion with the research project.

Co-operative Nuclear Research

In a written answer to a question in the House of Commons on March 7 as to the projects in which the United Kingdom is participating under the auspices of the European Nuclear Energy Agency, Mr. D. Freeth, the Parliamentary Secretary for Science, named the *Dragon* project at Winfrith Heath for the development of the high-temperature gas-cooled reactor and the Halden project in Norway for the development of a boiling-water reactor. The United Kingdom is also co-operating fully in the general work of the Agency through membership of specialist panels, committees and symposia. The *Dragon* project is managed by an international board, the United Kingdom representatives on which are appointed by the Atomic Energy Authority. According to the report for the year ended March 31, 1960, a start has been made on the construction of the experimental reactor, and Mr. Freeth understood that satisfactory progress is continuing. In reply to other questions, Mr. Freeth said that between 35 and 40 graduate physicists, drawn from physics departments in four universities, are making regular use of the facilities at the Rutherford Laboratory at the National Institute for Research in Nuclear Science, Harwell, and teams from two more physics departments would shortly be doing so. Present estimates indicate that the annual output of qualified scientists and technologists in Britain should reach 20,000 by about 1964, or after only eight years, instead of the 10–15 years recommended by the Scientific Man-power Committee of the Advisory Council on Scientific Policy.

Toxic Chemicals and Wild Life

In reply to a question in the House of Lords on March 9, the Joint Parliamentary Secretary to the Ministry of Agriculture, Fisheries and Food, Earl Waldegrave, said the attention of the Government had been directed to the report issued on February 22 by the joint committee of the British Trust for Ornithology and the Royal Society for the Protection of Birds on the effect upon birds and mammals of toxic chemicals used in agriculture. Comprehensive measures for dealing with this problem were agreed at a meeting last December convened by the Department, at which representatives of these two bodies were present, as well as delegates from the Council of Nature, the National Farmers' Union and the Association of British Manufacturers of Agricultural Chemicals. The measures agreed included a survey by the Ministry's pests officers of reported cases of unusual deaths among birds, foxes and badgers, and the results of the survey are to be reviewed with the interested organizations after this spring. The need for further research into the effects of the use of toxic chemicals in agriculture is being examined by a research study group appointed last year. While the Minister thought that these new arrangements would provide valuable evidence on which to base any further action which may seem necessary, he wished it to be widely known that the Government would welcome full information from naturalists or other interested persons about suspected poisoning of birds or mammals by toxic chemicals.

This reply did not entirely satisfy Viscount Elibank, Lord Hurcomb, Lord Shackleton and others, and points were pressed about the omission of any reference to possible dangers to wild life from the Ministry's 1961 list of approved chemicals, the desirability of independent inquiry and the effectiveness of co-ordination between the Ministry, the Agricultural Research Council and the Nature Conservancy. Lord Waldegrave said that he understood that an appendix to the notification scheme dealing with wild life is under discussion with the Nature Conservancy and other bodies, and he undertook to consider embodying a statement in the list itself in due course. Some concern was expressed as to whether sufficient research is proceeding, and Lord Hailsham, Minister for Science, agreed that co-operation could be improved and promised to consider issuing a statement as to the extent of research in progress. He did not, however, enter on the question of relations or co-ordination between the Agricultural Research Council and the Nature Conservancy, on which he was challenged by Lord Shackleton.

National Research Development Corporation of India

The sixth annual report and statement of accounts of the National Research Development Corporation of India covers the year ended March 31, 1960, in which fifty inventions were reported for development by eleven research institutions and individuals, bringing the total to 553, of which 397 are effectively in hand (Pp. 25. New Delhi : National Research Development Corporation of India, 1960). Thirty licences were negotiated and five processes went into production, making the total licensed and in production 37, with 51 licensed, but not yet in production. A new project on lightning arrestors has been sanctioned at the Indian Institute of Science, Bangalore, at an estimated cost of Rs. 1.60 lakhs. Installation of the pilot plant for phthalic anhydride at the Central Fuel Research Institute, Jealgora, is in hand and that for the de-ionization of cane juice at the National Sugar Institute, Kampur, has been installed. Pilot plant for white cement from felspar and a semi-commercial fluidized bed unit for textiles processing are being created.

New West Durham Borehole

At a recent meeting of the Geological Society at Burlington House, Piccadilly, Prof. K. C. Dunham, of the University of Durham, announced the results of a borehole that is being made at Rookhope in West Durham, and financed by the Department of Scientific and Industrial Research. Prof. Dunham, who has carried out extensive work on the metalliferous deposits of the Pennines, has for many years held the view that these continue to greater depths than have so far been exploited, and that they are related to an underlying granite mass. Support for the latter view has come from measurements of the variations in the gravity field by Dr. M. H. P. Bott, also at Durham. The borehole has now penetrated the 'hidden' granite 1,280 ft. below the surface, and has thus indicated that valuable minerals continue to three times the depth of local mining. The source of the minerals, nevertheless, lies at still greater depth, and the borehole is being continued.

Journal of Theoretical Biology

The first question one asks about this, as indeed about any, new journal is why is it necessary ? As the chief editor says in his preface, "it has frequently been difficult for an author to find an acceptable medium for theoretical papers" as contrasted with experimental ones ; and in this one must regretfully concur. Nevertheless, a journal devoted to theoretical papers over the whole of biology must, one would have thought, have had a unifying objective beyond that of providing an asylum for homeless brain-children. There is excitement abroad in biology to-day, just as there is excitement in cosmology, in nuclear physics, in theoretical chemistry ; and it is this excitement which, one feels, a journal of theoretical biology should catch, encourage and sustain. In the first issue there are several mathematical and physical papers which would leave the general biologist rather cold, a philosophical paper and an ecological paper which have little in them to excite the mathematician. There are, however, several papers of the kind one has in mind, dealing with the organization of cellular processes, one of which, by Dr. A. Szent-Györgyi, as might be expected from this author, being especially thought-provoking (*Journal of Theoretical Biology*, 1, No. 1. Subscription rates : (*A*) institutional subscribers, 121*s*. 6*d*. or 17 dollars ; (*B*) subscribers certifying that issues will be for personal use only, 86*s*. or 12 dollars. London and New York : Academic Press, Inc., 1961).

Kybernetik

We had occasion recently to take note of a new Russian journal which is being translated into English in Britain, concerned with cybernetics (*Nature*, 189, 795 ; 1961) ; our attention has now been directed to another journal, of similar scope, published in Germany, but edited by an international board. This is *Kybernetik*, published by Springer-Verlag of Berlin, and which is edited by a small group of people prominent in the field from Germany, Britain, the United States, Austria, France and the Netherlands. As with the Russian journal, the subject-matter in this relates to the transmission and processing of information and to automatic control processes, both as regards theoretical development within engineering and applications to models of biological functions. *Kybernetik* aims at cultivating experimental and theoretical work within the fields of information theory, control and automata theory, sensory processes and physiology of the nervous system as regards its information handling properties, and the like. The first issue of this journal appeared in January 1961.

New Pulsed X-ray Generator

The Zenith Radio Corporation announces the production of an X-ray generator which, it is claimed, produces 1-μsec. pulses of high-intensity radiation, with a repetition-rate of up to 30 per sec. The maximum operating voltage is 150 kV., with a current pulse of 130 amp., and a pulse power of about 20 MW. The radiation beam and the resultant radiographs are observed on a fluorescent screen and an image intensifier, which may be used for cine-radiography. The main use of the apparatus would appear to be in the field of industrial radiography under dynamic conditions. It can be used to study the internal structure of sealed vibrating systems which are not observable optically. In one demonstration the structure of a bullet in flight at 4,000 ft. per sec. was observed on a closed-circuit television system. Possible uses in crystal diffraction work under transient dynamic conditions are suggested. The claims for possible medical uses seem to be on less-firm ground. It is suggested that the very short pulses can give maximum radiographic information with minimum exposure of the patient. This appears to be based on a misconception that information depends on high-radiation intensity only, regardless of the total energy reaching the X-ray film. In one case, sufficient technical information is given for it to be approximately checked, and this raises doubts. It is claimed that adequate density on conventional film is obtained from a single pulse through 1·5 in. aluminium at 6 ft. Simple calculations from the figures given in the report show that such a procedure would involve a conventional radiographic exposure of 0·13 m.amp. and a radiation dose to the film of a fraction of a milliroentgen. Unless there are undisclosed factors, it is very difficult to imagine the production of adequate radiographs under these conditions. In spite of these questions, this new machine offers hopes of important advances in industrial radiography, probably mainly in the field of precision engineering.

Geology of Ben Nevis and Glen Coe

Since the first edition of the *Geological Survey Memoir* on "The Geology of Ben Nevis and Glen Coe" (Scotland : sheet 53) was published in 1916, the district has become somewhat of a national playground, much visited by tourists, mountaineers and parties of geological students. In the second edition (pp. 307, pl. xiii ; H.M. Stationery Office, 1960 ; 35*s*.), Sir Edward Bailey has rewritten and modernized his original publication, to provide a comprehensive account of the classical geological features of this fascinating area. Here one finds, associated with the famous cauldron subsidences, the most spectacular display of plutonic, hypabyssal and volcanic rocks of Lower Old Red Sandstone age known anywhere in Europe ; and the neighbouring Highland schists, with their recumbent folds and anticlines, have given rise to more disputes about structure and succession than any other group of British rocks studied in the past half-century. Although much of the memoir is difficult reading because of its content of local detail, the work will be warmly welcomed by the many geologists who frequent this instructive and scenic corner of Scotland.

Calendar of AGARD Meetings

The Advisory Group for Aeronautical Research and Development (AGARD) of the North Atlantic Treaty Organization announces the following schedule of meetings during April–August 1961 : Aero Space Medical Panel spring meeting (Lisbon, April 10–14) ; Flight Mechanics and Fluid Dynamics Panels, "Stability and Control" (Brussels, April 10–15) ; Flight Mechanics Panel, "*VTOL/STOL* Aircraft" (Paris, April 17–19) ; Ionospheric Research Committee, "Disturbances of Solar Origin on Communications" (Naples, May 15–20); Structures and Materials Panel Symposium on the Material Sciences (Paris, May 15–20); Fluid Dynamics Panel (The Hague, July 17–21); Avionics Panel Technical Meeting, "Microminiaturization" (Oslo, July 24–26); Structures and Materials Panel Specialists' Meeting of the Material Group (Oslo, July 24–26); Eleventh AGARD General Assembly, "Scientific Goals of Space Exploration and Research" (Oslo, July 27–28); Avionics Panel Technical Meeting, "Low Noise Electronics" (Oslo, July 31–August 2).

Methods of Teaching Undergraduates

THE University Grants Committee has appointed the following committee to make a comparative study of undergraduate teaching methods and practices current in the universities and colleges of Great Britain in the fields of arts and pure and applied science: Sir Edward Hale (*chairman*); Prof. R. G. D. Allen, professor of statistics, London School of Economics and Political Science; Dr. D. G. Christopherson, warden, Durham Colleges; Mr. J. S. Fulton, principal, University College of Sussex; Dr. J. H. E. Griffiths, lecturer in physics, University of Oxford; Prof. J. E. Harris, professor of zoology, University of Bristol; Prof. A. G. Lehmann, professor of French, University of Reading; Prof. M. McKisack, professor of history, Westfield College, University of London; Miss M. R. Price, headmistress, Milham Ford School, Oxford; Prof. E. M. Wright, professor of mathematics, University of Aberdeen; Mr. J. S. Morrison, lecturer in classics, University of Cambridge; and Mr. A. Tattersall, University of London (*secretary*).

Announcements

PROF. W. H. MCCREA, professor of mathematics in the University of London (Royal Holloway College), has been elected president of the Royal Astronomical Society, in succession to Prof. R. O. Redman.

LORD HURCOMB, chairman of the Nature Conservancy's Committee for England and a member of its Scientific Policy and Finance Committees, has been appointed chairman of the Nature Conservancy in succession to Mr. Arthur Bryce Duncan, who is retiring from the Conservancy after a long and distinguished period of service.

PROF. A. C. FRAZER, professor of medical biochemistry and pharmacology in the University of Birmingham, has been appointed a member of the Agricultural Research Council, in succession to Prof. K. Mather, who has retired.

PROF. RITCHIE CALDER, professor of international relations in the University of Edinburgh, has been awarded the Kalinga Prize by Unesco for his work for the popularization of science.

MR. R. G. MENZIES, Prime Minister of Australia, is to open a new worsted processing block at the Department of Scientific and Industrial Research's Wool Industries Research Association, Torridon, Leeds, on March 22. Further information can be obtained from Norman Manners, Chief Press Officer, Charles House, 5–11 Regent Street, London, S.W.1.

DR. NORMAN DENNIS NEWELL has been awarded the Mary Clark Thompson Medal of the National Academy of Sciences, in recognition of his outstanding contributions in the field of palæontology and geology, particularly concerning the ecology of modern and ancient calcareous reefs.

THE annual conference of the National Union of Teachers is to be held at the Dome, Brighton, during April 1–6. Further information can be obtained from Mr. F. Jarvis, National Union of Teachers, Hamilton House, Mabledon Place, London, W.C.1. The programme includes an address by Mr. H. D. P. Lee, headmaster of Winchester College, on "Sixth Form Studies", and one by Sir Wilfrid Martineau, chairman of the Education Authorities' Panel of the Burnham Committee, on "Thoughts on Higher Education".

THE Royal Photographic Society of Great Britain is organizing a three-day symposium on "Photography on Expeditions" at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7, during April 11–13. Further information can be obtained from Mr. A. G. Tull, Technicolor, Ltd., Bath Road, West Drayton, Middlesex.

THE spring meeting of the British Radio Spectroscopy Group will be held at the University of St. Andrews during April 6–7. The subjects for discussion are: electron spin resonance in free radicals (April 6) and nuclear magnetic resonance, including high resolution (April 7). Further information can be obtained from D. Bijl and F. A. Rushworth, Department of Natural Philosophy, The University, St. Andrews.

THE Società Italiana di Fisica is organizing an Enrico Fermi international school of physics at Varenna. Five courses are being held as follows: May 23–June 3, cosmic rays, solar particles and space research; June 19–July 1, evidence for gravitational theories; July 3–15, liquid helium; July 17–August 5, semiconductors; August 7–26, nuclear physics. Further information can be obtained from Prof. G. Polvani, Società Italiana di Fisica, Via Saldini, 50–Milano.

THE Institution of Chemical Engineers is holding a one-day symposium in the Chemical Engineering Department, University of Birmingham, on April 12. Among the subjects to be considered are: "Fabrication and Properties of Some of the Newer Constructional Metals" (Dr. N. P. Inglis); "Recent Developments in Wrought Corrosion Resistant Nickel-Base Alloys" (E. Warde); "Plastics in Chemical Engineering and Building" (S. Slaney); "Glass Fibre Wound Laminates" (Dr. F. F. Jaray); "Recent High Temperature Ceramics" (Dr. A. T. Green). Further information can be obtained from T. R. Bott, Honorary Secretary, Midlands Branch, Institution of Chemical Engineers, Chemical Engineering Department, The University, Edgbaston, Birmingham 15.

THE Road Research Laboratory of the Department of Scientific and Industrial Research is holding open days on May 3–4. The work of the Materials and Construction Division and of the Tropical Section will be on show at Harmondsworth, Middlesex, while the work of the Laboratory's Traffic and Safety Division will be on show at the new Road Research Track at Crowthorne, Berkshire. The Scottish Branch of the Laboratory at Thorntonhall, near Glasgow, will be open to visitors during June 7–8. Further information can be obtained from the Press Office, Department of Scientific and Industrial Research, 5–11 Regent Street, London, S.W.1.

THE NUFFIELD FOUNDATION

THE fifteenth annual report of the Nuffield Foundation, covering the year ended March 31, 1960, shows that the disposable income of the Foundation increased by £475,000 to £1,850,000. It welcomes the Charities Act of 1960 as making it easier, speedier and cheaper for a foundation to effect changes in its objects to suit modern needs, and emphasizes the importance of such freedom for the newer type of foundation, in an ever-changing social situation, concerned more especially with the future*. The modern foundation, it is emphasized, is profoundly influenced by the Rockefeller tradition of insistence on search for excellence and its belief that a foundation should evoke from a community a recognition of the need for a particular work the foundation has pioneered, followed by willingness to meet that need out of communal resources.

Of the allocation of £77,260 for science, a further £1,800 was for the work on natural selection and adaptation under Dr. E. B. Ford in the Department of Zoology and Comparative Anatomy, University of Oxford, where Dr. H. B. D. Kettlewell is working on the spread of black moths in manufacturing districts ; their invasion of the surrounding country has shown that resting moths are selectively eliminated by birds and has confirmed that industrial melanism is associated with a physiological superiority of the black moths. A further £4,080 over three years went to the Department of Zoology, University of Liverpool, for work on polymorphism in butterflies and the relation between human blood groups and disease, while £1,000 a year for five years was provided for the Biophysical Research Group then under Prof. A. L. Hodgkin in the Physiological Laboratory, University of Cambridge, for its work on the conduction of impulses in nerve and muscle, active transport or secretion of ions through membranes, and on muscular contractions. A grant of £5,700 over three years, covering the salaries of a senior research fellow and a graduate assistant, and the cost of materials, was made to the University of Liverpool for expansion of the work on the organic chemistry of porphyrins under Prof. E. W. Kenner ; an extended grant of up to £2,950 to the University College of Swansea is to enable Prof. H. E. Street to examine further the culturability of the excised roots of monocotyledonous species, and to undertake a biochemical study of the influence of light on the growth of wheat roots. Of grants totalling £20,000 in technology, £13,500 over five years to Loughborough College of Technology was to integrate the teaching staff with industry, with a further £500 for a study visit to the United States to make a critical analysis of the relation between academic education in colleges and practical training in industry of professional engineers.

Generally, the tendency during the year was towards larger grants, and grants totalling almost £310,000 in medicine included £62,500 over five years for a Nuffield Unit of Clinical Physiology, at the Postgraduate Medical School, Hammersmith Hospital, University of London ; £100,000 to the Zoological Society of London, to found the Nuffield Institute of Comparative Medicine, and create two senior research fellowships ; £60,000 (with a subsequent further £100,000) for a Nuffield Hearing and Speech Centre at the Royal National Throat, Nose and Ear Hospital ; £25,000 over five years to the United Oxford Hospitals for neurological research, including the development of new techniques, the building and maintenance of electro-physiological equipment, and work on the behaviour and structure of the nervous system when affected by disease ; and £18,000 for an extension to the Star and Garter Home for Disabled Sailors, Soldiers and Airmen, Richmond, Surrey. £5,000 over two years was offered to the Institute of Child Health, University of London, for research on biochemical abnormalities and mental retardation, and £6,000 over two years to Guy's Hospital Medical School for investigations into the biochemical basis of temporary psychoses.

In social research and experiment, for which grants totalling £432,000 were made, the Foundation's most important concern was the establishment of a national institute for social work, in accordance with the recommendation of the Younghusband Report, and for this purpose £250,000 was provided. A development fund of £50,000, available at the rate of £10,000 a year, was provided for the Institute of Race Relations, and £11,000 over three years for the School of Oriental and African Studies, University of London, for the first phase of a study of African customary law. Of grants totalling £308,000 in education, £50,000 was to Borough Road Teachers' College, Isleworth, for a science block, to be known as the Nuffield Science Building ; £130,000 to the University of Southampton for the Nuffield Theatre ; and £60,000 to the University of Leeds for the Nuffield Foundation Unit for the History of Ideas, under the direction of Dr. S. Toulmin.

Allocations for the care of the aged and research in ageing, totalling £140,000, included £122,500 to the National Corporation for the Care of Old People, and £7,100 for the Nuffield Gerontological Research Fellowship at the University of Birmingham. From the Oliver Bird Fund, £38,000 over five years was provided to the rheumatism unit at Edinburgh for a second research fellow, a pathologist, and secretarial and technical workers, while the Department of Experimental Pathology, University of Birmingham, received £22,500 over three years in support of studies on plasma proteins aimed at discovering an antiserum specific for rheumatoid factor as a whole, facilitating assay of the factor in human plasma and more precise localization in diseased tissues.

Grants for the Commonwealth overseas totalling £603,000 included £250,000 to the University College of Rhodesia and Nyasaland for a medical school ; £10,000 to the Forestry Department, Entebbe, Uganda, for work on the productivity of tropical high forest ; £20,000 towards the cost of the Library at Jan Smuts House, where the South African Institute of International Affairs is centred ; a further £7,500 to the Gayaza Girls' High School, near Kampala, to enable the diet side of the experiment on farming and balanced diet to be maintained ;

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* The Nuffield Foundation—Report for the year ended 31 March, 1960. Pp. xiii+144. (London : The Nuffield Foundation, 1960.)

£45,000 for a Nuffield visiting professorship at University College, Ibadan; £20,000 to endow a chair of anæsthetics at the University of Sydney; £40,000 for the recruitment of teachers of English in North Borneo; and £12,000 to establish a Literature Bureau in Sarawak. £25,000 to the Government of Mauritius was for an investigation into food production and marketing, and £4,200 to the University of Otago, Dunedin, and Canterbury Agricultural College for research into the lactation of sheep.

Allocations under the fellowships and scholarships schemes totalled £226,741. These included two new fellowship schemes in medicine, one of which will enable twelve family doctors practising in the United Kingdom to spend six months abroad during the next four years, studying subjects of importance in general practice. The other is for three types of fellowship in pediatrics. No medical fellowships or dental research fellowships were awarded during the year, owing to the absence of candidates of the desired quality, but one medical research fellowship and four fellowships and four scholarships in dentistry were awarded; three travelling fellowships for Civil Servants were awarded. Under the schemes for the Dominions and Colonies, five travelling fellowships in medicine, fifteen in natural science (and one fellowship in engineering from India) and nine in the humanities and social sciences were awarded, but only eight farming awards were made. Two travelling fellowships were awarded to Indian Civil servants. The Foundation's contribution to the Royal Society and Nuffield Foundation Commonwealth Bursaries scheme was renewed at £5,000 a year for a further five years, and twenty-one awards were made.

THE NATIONAL RESEARCH DEVELOPMENT CORPORATION

THE annual report and statement of accounts of the National Research Development Corporation for the year ended June 30, 1960, differs from the usual pattern, information on the position of development projects being mainly placed in an appendix, while the report itself deals at greater length with the Corporation's financial position after its eleven years of life*. The accounts, which are submitted in the form directed by the Board of Trade, with approval of the Treasury, show that annual revenue from exploitation continues to increase, and in spite of the increase in administrative expenses since 1957, the total income of £316,441 almost balanced the total outgoings of £328,961, apart from external development expenditure of £427,316 and interest of £132,096 on Board of Trade advances. The report suggests that the development projects overall, regarded as assets, may in the event provide a greater return than their value written down in the accounts, but in view of the risks involved the Corporation considers it would be unwise to assign to them a higher value at present. It would be easier to present a picture of the success of the Corporation's development activities if development expenditure remained stable for, say, 15 years, but in fact it has increased substantially from £112,988 in 1954–55 to £427,316 in 1959–60, plus forward commitments amounting to £739,000, and the balance sheet thus probably gives an unduly pessimistic picture of the financial position of the Corporation. Future royalty earnings are also expected to be considerably greater than the asset shown, but can only be assessed on a statistical basis from past experience. Meanwhile, interest on the Board of Trade loans continues to grow, and since the Corporation borrows at fixed rates of interest to cover long-term 'risk' ventures at a level of about double its revenue earnings, by the time returns are received on successful projects, the accumulated interest on borrowings to cover both successful and unsuccessful projects will be a formidable sum.

For all these reasons it is still extremely difficult to predict when the Corporation will be able "to secure, in so far as can be done consistently with the fulfilment of their purposes that the return to them from their activities shall be sufficient to meet their outgoings on revenue account". It seems that it may not be possible to recover enough profit from successful ventures to cover both accumulated interest on loans and losses on unsuccessful projects. Meanwhile, in a written answer to a question about the report, the President of the Board of Trade stated in the House of Commons on January 24 that he had asked the Corporation to consider with his Department the possibility of devising some means of ascertaining more readily what the true financial position is, and in particular of assessing the Corporation's ability to commence payment of interest on the monies advanced to it.

In its exploitation activities, the Corporation completed 81 licence agreements in the United Kingdom during the year as against 61 in 1958–59, and 456 such agreements were in force at June 30, 1960. Royalties and other payments under licence and option agreements amounted to £259,000 compared with £182,000 in the previous year, about half these receipts being derived from overseas business. At the end of the year the Corporation held 597 United Kingdom and 1,015 overseas granted patents and 469 United Kingdom and 1,015 overseas patent applications. Of the 1,021 inventions communicated to the Corporation during the year (compared with 681 in 1958–59), 301 were from Government Departments and Research Councils; 44 from Commonwealth official organizations, 137 from universities, 20 from research associations and 8 from charitable organizations. Private firms and individuals in the United Kingdom contributed 478, in the Commonwealth 15, and elsewhere overseas 18. Of the 255 patent rights assigned, 170 were from Government Departments and Research Councils, 70 from universities, and 11 from industrial research associations.

By the end of the year the Corporation was providing financial assistance for 35 development projects covering a very wide technical field, and some of these, such as hovercraft and fuel cell developments, are of a major character and may involve further large capital commitments. Among the new projects for the year are one for the development of electronic automatic equipment for process plant control, Prof. D. Gabor's project for the thermionic generation of electricity, one for the design of an infinitely variable speed gear, and the development of interferon, a substance which has the important property of interfering with the reproduction of normal virus and may be of wide utility against virus diseases. Major revenue earning inventions during the year include cephalosporin *C*, tri-iodothyronine, the *Emidec* 2400 electronic computer, Prof. Wain's selective weedkillers, the Hutchinson–Scarrott pulse height analyser, hecogenin, the carbon monoxide detector, an insecticidal fumer, and a microdensitometer. The Joint Development Committee, set up with the Department of Scientific and Industrial Research in 1958 to examine development problems of common interest and advise on the way in which particular development contracts should be handled, has considered and advised on some twenty proposals for research and development during the year; it has proved a useful forum for considering matters of interest to both bodies, ensures that overlapping is avoided, and facilitates the investigation by both bodies of gaps in research and development in both fields.

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* National Research Development Corporation. Report and Statement of Accounts for the year 1st July, 1959, to 30th June, 1960. Pp. ii+80. (London: H.M. Stationery Office, 1961.) 1s. 6d. net.

ADVISING THE FARMER

WITHIN the Agricultural Education Association, a new section, the Advisory Methods Section, has recently been formed, and a series of papers were read at a meeting of this section in London on December 8. The general topic was to be a review of investigations in advisory methods: in practice, only one of the three speakers dealt at any length with research which has already been done, though all were concerned to analyse the advisory process with a view to further research.

Mr. T. Moore, of the Ministry of Agriculture in Northern Ireland, was the first speaker. He began by discussing the ways in which advisers could be most useful to farmers, and gave it as his view that 'trouble-shooting' was not of great value, by comparison with the giving of advice on the broader topic of farm management. This point of view is not uncommon among farm advisers, though the idea that the person providing the advice is better fitted to say what topics he should advise on than the person seeking the advice seems to have some unfortunate implications. If for no other reason, the adviser may well have to appear willing to do some 'trouble-shooting' if he wants to get invited on to certain farms, and successful 'trouble-shooting' in such circumstances may serve to win the farmer's confidence.

Using an arbitrary classification of farmers into three categories, Mr. Moore pointed out that advisers themselves can often learn a lot from the best farmers, that there will be a second group who cannot be helped much, leaving a third group consisting mainly of hard-working farmers who farm mainly by traditional methods. Farmers in this last group are those to whom advisers can be of most use. He claimed, too, that the size of his farm is not a satisfactory indication of how receptive a farmer will be to advice, though he gave no statistical data to support his claim. Without such evidence, the claim seems an unlikely one, for nearly all studies of farmers have shown that acreage is by far the most useful criterion for assessing the progressiveness of a farmer.

Commenting on advisory methods, Mr. Moore quoted some Australian data which stated that farmers visit other farms a good deal, and he thought this emphasized the importance the farmer attaches to seeing a technique in operation. Another interpretation of the same data would be that farmers feel that they can obtain an unbiased point of view in discussion with a practising farmer. Mr. Moore also commented on individual advisory visits to farms, and remarked that some people were somewhat sceptical about their value. He felt that pilot farms were useful, particularly where the farm chosen is not very different in size from other farms in the district, and claimed it as essential that the pilot farmer farms at a profit, regardless of the appearance of the farm. There seems to be some degree of conflict here with theories of leadership, since farmers commonly appear to have a favourable opinion of farmers who operate on a larger scale than themselves, though there are also data suggesting that the difference in scale must not be too great.

Mr. A. W. van den Ban talked about research on advisory methods in the Netherlands, where there are now five rural sociologists engaged in such work, two of them doing this as a full-time occupation. This certainly compares favourably with Great Britain, in which there is not even one such person who is employed to do full-time research in rural sociology. Mr. van den Ban discussed the part that different sources of information play in the adoption process, with the mass media coming in at the early stages, followed by 'individual' sources, such as talking with advisers, or with other farmers. He recommended that the adviser should manipulate the sources of information at his disposal so as to ensure that the farmer receives information in this way: in practice, because the mass media normally reach a farmer before the 'individual' sources, this does not seem to be any guarantee that this is the ideal way.

Dutch farmers were, until fairly recent times, on a subsistence economy and this still affects their way of thinking. When a whole group of farmers feel this way, it is made even more difficult for an individual to be progressive, and even such activities as reading a book or visiting a meeting in the daytime may be frowned on by the community.

Mr. van den Ban discussed the various sources of information, including the mass media, which a farmer can readily ignore since advice cannot be adjusted to individual needs, as well as farm visits, usually made by an adviser who lives among the farmers he serves, and who does not have so much more training than the farmers: this may mean that there is no great difference in status between the local adviser and the farmers, a fact which should make communication easier. Psychologists have been giving training courses to advisers, in an attempt to help them to cope with problems of inter-personal relationships, for example the somewhat delicate situation which may develop when a young adviser has to tell an experienced farmer, older than himself, how to correct his mistakes.

In the Netherlands, it has been found that most of the progressive farmers have visits from advisers, whereas the traditional ones are less likely to be visited. This is not necessarily bad, as Mr. van den Ban explained, since the traditional farmers may learn from studying what the progressive farmers do. When discussing this, and the more deliberate attempts to promote this type of diffusion process, using pilot farms, Mr. van den Ban pointed out that this mechanism may not operate if the farmer chosen is regarded by others as one who is so skilful, or has such good land, that he is in a different category from themselves. If this occurs, this farmer's example may be ignored because it is felt to be irrelevant to their type of farming.

Mr. van den Ban discussed how farmers who are friends appear to influence each other so that, for example, they are very likely to have the same brand of milking machine. He quoted data to show that farmers felt that attending group discussions had a greater influence on them than attending lectures, and cited data from the United States which appeared to point to the same conclusion. He seemed not to be aware that this American finding has been to some extent discredited by more recent research. Mr. van den Ban concluded by emphasizing the need for directing advisory methods to changing the aims of the community, rather than the aims of individuals, so as not to set the individual in conflict with the community norms.

Mr. H. Burr, of the National Agricultural Advisory Service, gave the last paper. Mentioning the frequently occurring finding that farmers are much influenced by other farmers, Mr. Burr wondered if this could be an artefact due to farmers feeling least damage to their self-esteem by admitting to influences of this kind. This is a fair criticism since these research claims are based on farmers' own reports of what influenced them, though there has been some research recently which shows that farmers will go to a lot of trouble to visit farms where a new technique is being tried, before trying it themselves. With the technique concerned, it seemed likely that a talk with the farmer concerned was being sought, rather than an attempt to learn by seeing the technique in action.

Mr. Burr noted that the National Agricultural Advisory Service as a body strongly favoured the individual advisory visit, but said that some within the Service were trying to 'personalize' group advisory methods, so as to achieve the advantages of both. Mr. Burr also talked about 'leaders', and ways of designating them: self-designation may be misleading and direct-designation, for example, when a pilot farm is chosen by the adviser, has its dangers if the farmer concerned is so different from other farmers that they cannot 'identify' themselves with him. Indirect-designation occurs when farmers are asked to name their leaders, though it is not always easy to be sure that the choice of a farmer whose farm is to be used as a pilot farm, or for a farm walk, does not influence other farmers to regard such a person as a leader. Mr. Burr also pointed out that there are few data to show how much influence such leaders have, nor is it known whether the same farmers are the leaders over a long period. He felt that more should be known about this before the Advisory Service commits itself to using leaders deliberately as an advisory technique.

A lively discussion followed these papers and showed the interest which exists in problems of this type, though, clearly, as the speakers emphasized, much more research is needed. The meeting was, in fact, a most interesting one, and it is to be hoped that this Section of the Agricultural Education Association will sponsor many more. It is heartening to think that a group of people, one of the main tasks of which is to encourage a greater use of scientific advances, should be so concerned to evaluate their own methods of passing on these ideas. D. SHEPPARD

SWISS SOCIETY FOR NATURAL SCIENCES

THE one hundred and fortieth annual session of the Swiss Society for Natural Sciences was held in Canton Aargau during September 23–25, 1960. By a lucky coincidence the main exhibitions connected with the quincentenary of the University of Basle had been extended and were still on view nearby, including the unique Greek treasures and the remarkable display of the University's history, as well as the more purely scientific attraction of the *Oreopithecus* exhibits and the gorilla baby, 'Goma', born in Basle Zoo.

The Aargau meeting opened with a visit to the installations of *Reaktor AG* in a large forest clearing at Würenlingen. Here the first Swiss atomic reactor, *Sapphyr*, originally shown at Geneva, and a second larger reactor are already available for industrial and research purposes in association with Zurich Technical High School. A third reactor, to be larger again, is in process of construction.

The first general assembly was held in the twelfth-century town of Zofingen, cradle of the nation-wide student society Zofingia since 1819, which has been much enlivened by the colourful quinquennial celebrations. Here the central president, Prof. G. Tondury (Zurich), in his address of welcome introduced the opening speech of the annual president, Dr. K. Baeschlin (Aarau), on the main theme of the session, "Der Mensch und seine Beziehungen zur Umwelt". The subject fell into four categories: man and the universe; man and Nature in a geographical sense; man and animal; and man and man. The first two were treated by Prof. M. Waldmeier of Zurich ("Die Beziehung Mensch-Weltall"), and Prof. L. Emberger of Montpellier ("La Relation Homme-Nature") at the first general assembly. The second two, by Prof. O. Koehler of Freiburg in Breisgau ("Die Beziehung Mensch-Tier"), and Prof. W. Loffler of Zurich ("Die Beziehung Mensch-Mensch"), were given at the second general assembly, in the now State-owned Castle of Lenzburg, once (1892–1911) the possession of Lady Mildred Bowes-Lyon and her American husband, Aug. E. Jessup, to whom is due the present good condition of the property, maintained after them (1911–56) by the last private owners, the family of Lincoln Ellsworth, the arctic explorer in partnership with Amundsen.

All four lectures will appear in full in the *Acta* of the Society. Each began with a brief historical survey and underlined lessons to be learnt from the past, but the emphasis was on new knowledge, and some particular aspect was focused in each case. Prof. Koehler illustrated by striking film-sequences the often disregarded capacity of animals to learn, with extreme rapidity, and without direct guidance from man. Prof. Loffler, who had been deliberately chosen as an eminent medical practitioner in preference to a psychiatrist for the subject of human inter-relations, had naturally some words of warning on present-day ethics in his profession, and lent the conviction of intimate experience to the plea for better family relations which both mental and physical welfare specialists endorse to-day, besides surveying the field of modern mass-problems of over-ageing, over-population, hunger and over-production which science must help to solve. Prof. Emberger was inclined to stress the destructive influences of man on Nature more than the work of reclamation by his illustrative slides on this urgent topic of the day. Prof. Waldmeier could not but give much place to modern developments in space-research and the formerly unthinkable extension of experimental techniques to astronomy.

Some of the sectional proceedings were designed to fit into the same general theme of "Man and his Relations to his Environment". A symposium on statistical methods in biology and medicine, organized by the Swiss Society for Medical Biology, in conjunction with the Mathematical Society and the Society for Logic and Philosophy, had for its topics: development and prognosis of the death-rate in Switzerland (P. Nolfi, Zurich); development of the cancer death-rate, with special reference to lung cancer (H. Wiesler, Zurich); medicine and statistics (E. Tanner, Zurich); medicine and logic (E. Batschelet, Basle); optimal information by use of statistical methods (L. Le Roy, Zurich); statistics as an aid to medical research (D. Högger, Zurich).

The medical and psychological scene was prominent also in the historical section, notably in a contribution by Madame A. Virieux of Rolle, "Quelques Remarques à propos de la Psychologie à l'Antiquité", in which she pointed out, among other things, that Plotinus's theory of a two-fold memory—one purely spiritual, one body-linked—influenced Bergson; that the contrast between Socrates, who believed emotion to be entirely governed by reason, and Aristotle, in his recognition of the intimate union of body and soul, is of great relevance to modern psycho-somatic theory. Anxiety as treated by Aeschylus anticipates Freud. The fact that Plato, in his extensive study of vision, took colour to be a subjective phenomenon, in the sense of an original product for each individual of the complex visual process, seems as striking as the subtle and still little-understood fusion he attempted (probably in order to explain the redressing of inverted images) of the two opposing theories of currents emerging from the eye and the object respectively, as the basis of that process.

Psychological and social influences were touched on in a context of recent mathematical history by Dr. R. C. H. Tanner (London) on "W. H. Young et la Méthode des suites monotones", with reference to Young's anticipation by Henri Lebesgue in extending the scope of the Riemann integration process. This discovery was incidental to a much wider field of research for Young, and the setback cannot have made on him the emotional impact assumed by his English biographers, while the role attributed to it in the lack of recognition he received from British appointments boards during his life-time can only, if admitted, impugn the 'cult of results' and lack of methodological insight then common in England; an offshoot perhaps of a competitive and sporting tradition that dies hard.

The mathematical section in its own right made a relatively modest show, because, evidently, of the special effort during June 25–26 in celebrating the Swiss Mathematical Society's half-centenary at Zurich, when the following contributions were made: Prof. M. Plancherel (Zurich) on "Mathematiques et Mathematiciens en Suisse (1850-1950)", Prof. H. Hopf (Zurich) on "Einige neuere Ergebnisse in Differentialgeometrie und Topologie", Prof. G. de Rham (Lausanne) on "Quelques Notions de Base de la Theorie des Variétés Intégrables"—the last two coincided with the international colloquium on "Differential Geometry and Topology", held under the auspices of the International Mathematical Union during June 20–25. At a special ceremony, the topologists Prof. H. Whitney (Princeton), Prof. J. Leray (Paris) and Prof. G. de Rham were honoured and given commemorative scrolls by the annual president of the Swiss Mathematical Society, Prof. H. Jecklin (Zurich).

The physical section concentrated largely on nuclear and allied research; the chemical section also had on its list one paper on the role of heavy metals in the mechanism of radiation damage and shielding (W. Brintzinger, Basle), but for the rest it devoted itself mainly to more classical synthetic and reaction chemistry.

The section for geophysics, meteorology and astronomy heard Prof. Jean Lugeon, head of the Swiss Meteorological Station, Zurich, report on radiosonde measurements in connexion with the industrial air-pollution at Aigle, which he has been actively campaigning against in recent years. J. Riecker, of the same Station, spoke of sunrise in the ionosphere and its repercussions on long-wave propagation. G. Barnes, an Australian who is temporarily at the Technical High School, Zurich, discussed the prevention of water evaporation by surface films. There were also reports on the expedition to El Aaiun in the Spanish Sahara for the total eclipse on October 2, 1959 (Prof. M. Waldmeier of the Federal Observatory, Zurich), and on the unusual fluctuations in the ozone content at different heights in the stratosphere during the sudden heating up in January–February 1958 (H. Dütsch, Zurich). Other communications concerned rain and ice, and climatological problems. In the botany section, the items of special local interest were outweighed by the research work of general import of the strong Geneva school, with F. Chodat and others, and two obituary notices: "W. Vischer 1890–1960", a promoter of the Swiss Society for the Protection of Nature, by M. Geiger-Huber (Basle), and "Eduard Rubel 1876–1960", by W. Lüdi (Zurich).

Two recent international congresses were reported by members who had taken part: that on "Logic, Methodology and Philosophy of Science", at Stanford, by E. Walter (Zurich) and I. M. Bochenski (Freiburg), and that on "Geography", at Stockholm, by E. Schwabe (Berne). R. C. H. TANNER

HEALTH IN INDUSTRY

IN a foreword to a publication issued by the Ministry of Labour, the Minister, Mr. John Hare, directs attention to the fact that more than 300 million working days are lost each year in Great Britain because of sickness or injury.

The Minister's foreword is contained in the booklet, *Health at Work**, which gives fourteen examples of individual industrial health schemes operating successfully in factories in different parts of Great Britain, and the Minister hopes that these examples will encourage other firms to follow suit. The booklet is being widely distributed to factory occupiers by H.M. Factory Inspectorate. It deals with such questions as how factory medical services are organized, and what they achieve, the attitudes of workers to their introduction and the advantages derived by both employer and worker.

In carrying out the survey, the Ministry obtained from each factory details of the nature of the work done there, the organization of the medical services, the work of the medical officers and nursing staff, and the cost of each scheme. Four of the factories employ from 231 to 300 workers; five employ from 500 to 1,000; three from 1,000 to 5,000, and two more than 5,000. Included in the industries represented are heavy engineering and foundry work, copper and metal refining, the manufacturing of electric cookers, refrigerators, electro-plated ware, carbon black, printing machinery, animal feeding stuffs and vegetable oil. Also included are wool and worsted spinning, the processing of asbestos yarns and pipes, and the preparation of insecticides, fungicides, fine chemicals and pharmaceuticals.

Fear about costs is suggested as being the biggest single factor in preventing a more rapid spread of industrial health schemes. The booklet gives detailed figures of the cost of the schemes provided. The annual running costs vary widely according to the nature of the services provided and the numbers employed. Of the 14 schemes described, seven cost £2 or less a head per annum, five cost between £2 and £3, and the other two more than £3. The booklet also mentions an attempt at another factory to weigh in terms of cash the worth or value of such a scheme. This led to the conclusion that, on a conservative estimate, the annual recurring costs of the medical scheme were more than covered by savings in time lost, and that the long-term benefits in better health and morale were a bonus alike to the company and the employee.

It is emphasized that the booklet does not intend to provide a ready-made pattern from which an employer can pick a scheme to suit his pocket, or the size of his factory and his business. What it does show is how, in practice, such schemes are operating to meet particular needs of particular firms, and the considerable variety of functions which the services can perform to the advantage of employers and workpeople. The reasons why firms considered it worth while to spend more money voluntarily on medical supervision varied according to the type of industry, but the following were typical: (1) the pre-employment examination was a safeguard to management and workers; (2) from a practical point of view, speedy attention to accidents and ill-health saved time; (3) improvement in relations between employers and workers resulted from such a scheme.

The general impression drawn from reactions on the part of workers was that schemes of medical supervision described in the booklet provided a personal service which the workers greatly appreciated. The fact that immediate and expert attention could be given to the injured and sick aroused a feeling of confidence among them. The booklet indicates that in one or two instances there was some initial suspicion on the part of workers which soon disappeared with experience of the working of the schemes.

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* Ministry of Labour. *Health at Work: a Description of Medical Services in Fourteen British Factories.* Pp. 76. (London: Ministry of Labour, 1960.)

ELM DISEASE

ALTHOUGH the elms are not, nowadays, regarded as important species in forestry proper, the Forestry Commissioners sometimes feel that it is incumbent on them to devote time and money to conduct research into problems concerned with trees in which the British public as a whole are generally interested. A recent publication*, which would have been more correctly described as referring to southern England, deals with such a case. It is concerned with a fungous disease of elms caused by *Ceratostomella ulmi* (Schwartz) Buis., which was first recorded in France in 1918, and in Holland in 1919, and is suspected as having been introduced with elms imported from eastern Asia. It soon became widespread in northern Europe and in North America.

It was first recorded in England in 1927, by which time it was giving much concern to tree-growers in Holland, where its severity at one time earned for it the title of the Dutch elm disease. The effect of the fungus is to cause a vascular wilting of the leaves followed by the death of twigs and branches and even of whole trees. The unsightliness of the dead trees must have attracted the attention of all who travel through those counties where elms are such a notable feature of the landscape. The disease was probably present in Great Britain before its first recording.

The task of studying this disease and of keeping an eye on it was entrusted by the Forestry Commission in 1928 to Mr. T. R. Peace while he was stationed at the Imperial Forestry Institute, Oxford, and he has continued to do this since his appointment to the staff of the Commission, where he is now chief research officer. The present publication, issued by the Forestry Commission, is based on his work, and especially on surveys undertaken by him, during the 31 years up to 1959, but he also acknowledges his debt to American workers in Great Britain in the same field.

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* Forestry Commission. Bulletin No. 33: The Status and Development of Elm Disease in Britain. By T. R. Peace. Pp. iv+44+76 plates. (London: H.M. Stationery Office, 1960.) 10*s*. net.

This disease is one in which there is a very intimate connexion between two organisms, one from the plant world, the other from the animal world. In 1931 this was established by Fransen, who found that bark beetles of the genus *Scolytus* were carriers of the fungus, which can also enter the tree through wounds and especially through the roots, which, from the suckering habit of the English elm, must be not uncommon.

The surveys revealed that the disease reached its peak in 1931, and that it has steadily declined since 1936–37, not only in the number of elms attacked but also more especially in the severity of the damage caused. It is suggested that the war-time clearance of diseased elms, made possible by the opportunity to market the timber, may have led to a reduction in the beetle population and to infections due to it. It is estimated that the total loss due to the disease lies between 10 and 20 per cent of tree elms, and that this is being made good by sucker growth. Two lines of action were taken to combat the disease. In Holland and the United States a policy of widespread felling of diseased trees was adopted ; in Great Britain things were left to run their course. In view of the considerable degree of recovery of diseased trees, in support of which the author provides many good plates, he is perhaps justified in concluding that the loss of elms would have been much greater in Britain if the first policy had been adopted. He advises the awaiting of the field trials of strains resistant to the fungus before widespread planting of elms in hedgerows or for amenity is undertaken. The disease may long continue as a minor nuisance, but is not expected to bring about the disaster of complete annihilation of the elms immemorial. The great weakness of the investigation undertaken by Mr. Peace is one that is associated with most of the publications of the Forestry Commission, namely, lack of appreciation of the importance of allowing for locality differences. The data in respect of both climate and soil are quite inadequate for the treatment of such an exacting genus as *Ulmus*. When a study is allowed to range over a wide variety of site conditions, it is essential that it should be handled in such a way that site variation is allowed for.

M. L. ANDERSON

PHOTO-EMISSION FROM METAL SURFACES MEASURED WITH GEIGER COUNTERS

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DURING the past several years, J. Kramer has investigated a group of phenomena involving the emission of low-energy electrons from solid surfaces. The findings are summarized in two of his papers[1]. This work by Kramer, more recently extended by others, provides an interesting and sensitive new method for investigating the surface condition of both metals and non-metals. Through studies of this so-called Kramer effect, some insight has been gained into a number of phenomena, such as the delayed emission of electrons ('exo-electrons') that occurs when certain non-metals (for example, aluminium oxide powder and gypsum) are crushed or irradiated ; the dependence of photo-emission on surface treatment of metals ; and electron emission caused by heating below the temperature of thermionic emission. For purposes of theoretical interpretation, and determination of possible practical applications, it is essential to know whether these phenomena are inter-related, and to what extent. The purpose of this article is to report some preliminary experimental observations obtained during an investigation of these phenomena in our Laboratory that we believe are pertinent to this problem.

The emission of low-energy electrons from bulk specimens of several metals of commercial purity (aluminium, tin, copper, nickel and zinc), before and after various mechanical treatments (abrasion, filing, polishing and compression), was measured by means of windowless Geiger-flow counters at room temperature. The specimen, in the form of a disk, ¼ in. thick and 1 in. in diameter, formed a part of the cathode ; in one of the counters with a fused quartz envelope, the specimen constituted the largest part of the cathode. The distance of the anode 'point' of the counting tube from the specimen was ¾ in. The tubes were operated in the voltage-range of 1,200–1,300, where the extent of the plateau was at least 150 V. The flowing gas, at atmospheric pressure, consisted essentially of helium (about 98·5 per cent) and contained *iso*butane as the quenching agent ; a small amount (about 0·05 per cent) of water vapour was detected in this gas by mass spectrometry. The background radiation during these measurements was determined to be about 25 counts per minute.

Contrary to the findings of some investigators[2], we detected no emission of electrons from the freshly abraded, filed, or compressively strained metal specimens when the Geiger counters were made completely light tight. In other tests we have made with a quartz-envelope Geiger counter, emission was detected only when the light shielding was not adequate. Evidently, the intensity of stray light needed to induce photo-electron emission from mechanically treated metal surfaces is extremely low. Thus it appears that true delayed emission of low-energy electrons (exo-electrons) does not occur in the absence of any stray light during the detection phase of the experiment.

The blackening of photographic emulsions in contact with freshly deformed or abraded metal surfaces (the so-called Russell effect) is known to result from the formation of hydrogen peroxide at

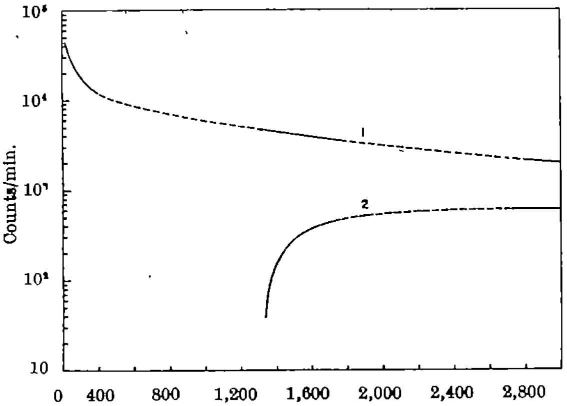

Fig. 1. Photoemission from filed tin surface before and after irradiation. (1) Decay curve of filed tin surface; (2) recovery curve of filed tin after 1-min. irradiation at 50 kV. and 40 m amp. A tungsten target X-ray tube was used. This sample had reached the decay-level of that in curve 1 at the time of irradiation.
Note: Broken lines indicate intervals during which no readings were taken

the metal surface. Grunberg[3] has associated the formation of hydrogen peroxide, in the presence of water vapour and oxygen, with the emission of low-energy exo-electrons. The macroscopic aspects of both the Russell effect and exo-electron emission have been rather thoroughly investigated. Recently, however, Meleka and Barr[4] have applied an autoradiographic technique to a study of the microscopic features of the Russell effect. They found that high-resolution photographic emulsions applied to surfaces of statically strained, high-purity zinc, single-crystal rods were blackened in microscopic areas (lines or rows of dots) corresponding to slip lines associated with varying amounts of slip displacement. In addition, they suggest that exo-electron emission from plastically deformed metals also originates from slip bands on the surface. In view of the fact that we have been unable to detect electron emission from abraded or deformed metal surfaces in complete darkness, it seems to us that the mechanism of the Russell effect cannot be explained by the action of delayed electrons detectable in a Geiger counter.

It is well known that X-ray irradiation of certain non-metals, such as aluminium oxide powder, may induce true delayed electron emission. We have confirmed this observation, and, in addition, have found that the intensity of the delayed electron emission is closely associated with the amount of water contained in the aluminium oxide powder. The emission from samples containing 3 per cent water was about 50 times more intense than that from samples containing about 1·5 per cent water. A similar connexion between electron emission and adsorbed water, during heating and cooling of quinine sulphate, has been reported by Kramer[1].

Our experiments on the X-ray irradiation of metals have demonstrated that such treatment not only does not induce delayed electron emission, but also strongly de-activates the materials with respect to photo-emission. The curves of Fig. 1 show the effect of X-ray irradiation on the photo-emission of a filed tin surface. Other metals have exhibited similar behaviour, with a varying rate of recovery of photo-emission. There is a formal similarity between the 'fatigue' caused by intense light and this de-activation by X-ray irradiation; however, the X-ray fatigue seems to be a more complex phenomenon. Further work is planned to investigate this de-activation by irradiation and the recovery of photo-emission in different atmospheres.

Photo-electric wave-length thresholds were determined by means of the counting arrangement previously described, and a grating monochromator and an incandescent ribbon lamp as a light source. The thresholds were found to be in the near ultraviolet region of the light spectrum, and they differed slightly for the different metals. Table 1 shows the estimated values of these thresholds, together with those reported in the literature for the same pure metals in vacuum.

Table 1. PHOTOELECTRIC THRESHOLDS

	Filed		X-irradiated		Pure metals in vacuum (ref 5)	
	Wave-length (Å.)	eV.*	Wave-length (Å.)	eV.*	Wave-length (Å.)	eV.*
Al	4000	3·07	3500	3·51	2950	4·20
Zn	3600	3·41	3300	3·72	2900	4·27
Sn	3500	3·51	3200	3·84	2820	4·39
Cu	3000	4·09	2900	4·24	2770	4·48
Ni	3200	3·84	3000	4·09	2520	4·91

* Electron volts.

These wave-length thresholds follow, in general, the same order as that for pure metals in vacuum, and differ from them by several hundred Ångström units. No selective maxima in the visible part of the spectrum were found, which agrees with the observations of Sujak[6].

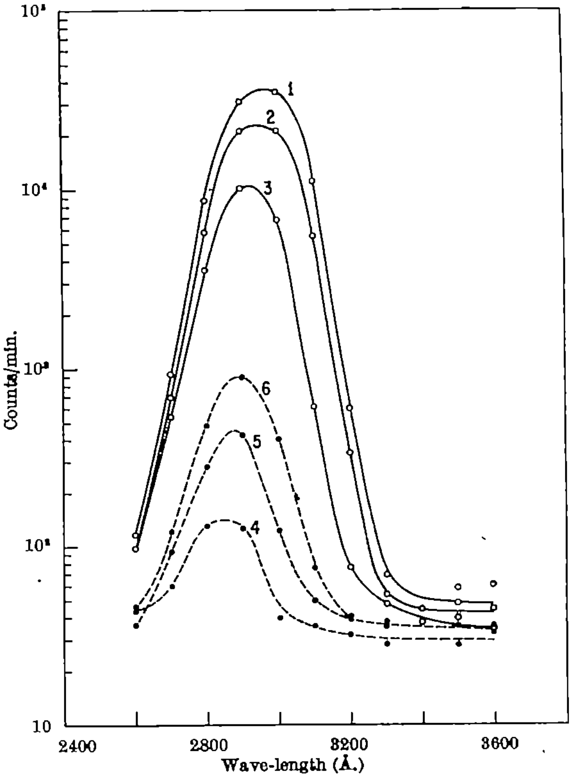

Fig. 2. Decay curves from filed tin, before and after irradiation (1) Immediately after filing; (2) 50 min. after filing, (3) 1,100 min. after filing; (4) immediately after 1-min. irradiation at 50 kV. and 40 m.amp., tungsten target X-ray tube; (5) 35 min after irradiation; (6) 400 min after irradiation
Note: Each point is an average of two readings, symmetrically spaced with respect to the beginning and the end of the run, to minimize the effects of light fatigue

The decay and recovery curves for photo-electric emission from tin, obtained at various intervals after mechanical treatment, are shown in Fig. 2 as a function of wave-length. The maxima in the curves are a result of the combined effects of: (1) a rapid decrease in intensity of the light from the incandescent source with decreasing wave-length, and (2) the rapidly increasing photo-sensitivity with increasing wave-length. These maxima provide a sensitive, qualitative criterion for a shift of photo-sensitivity with change of wave-length. A shift of the maxima of up to 200 Å. was observed in some metals because of differences in surface condition, that is, abraded, irradiated, or after subsequent recovery.

Results such as shown in Fig. 2, as well as other results obtained with a mercury arc light source in the shorter wave-length region of the spectrum, although not yet conclusive, may contribute to an understanding of the increased photo-emission yield resulting from mechanical treatment of metal surfaces. Of particular importance is the question of whether this increase is caused solely by a lowering of the work function of the metal surface. The role of adsorbed gases and oxide films on the metal surface is also of interest.

[1] Kramer, J., *Z. Phys.*, **133**, 629 (1952); *Acta Phys. Austriaca*, **10**, 20 (1957).

[2] For example, Von Voss, W. D., and Brotzen, F. R., *J. App. Phys.*, **30**, 1641 (1959), footnote

[3] Grunberg, L., *Proc. Phys. Soc.*, B, **66**, 153 (1953).

[4] Meleka, A. H., and Barr, W., *Nature*, **187**, 232 (1960).

[5] Simon, H., and Suhrmann, L., *Der Lichtelektrische Effect und seine Anwendungen*, second ed. (Springer, Berlin-Göttingen–Heidelberg, 1958).

[6] Sujak, B., *Phys. Ber.*, **15**, 209 (1959). Sujak-Grunberg, *Brit. J. App. Phys.*, 10, 102 (1959).

ACTIVE SENSITIZATION TO TUBERCULIN WITH CELLS FROM TUBERCULIN-SENSITIZED GUINEA PIGS

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THE cellular transfer of tuberculin hypersensitivity in guinea pigs was first demonstrated by Chase[1]. Transfer with disrupted peripheral blood leucocytes has been demonstrated in man[2] but not in guinea pigs[3]. After passive transfer, to non-sensitized animals, of live cells from guinea pigs hypersensitive to tuberculin, the recipient may show skin hypersensitivity to tuberculin for 3 months or more. This might be explained by survival of the donor cells or by active sensitization of recipients superimposed on the passive hypersensitivity transferred with the donor cells. The following experiments indicate that the second explanation is probably the true one.

Guinea pigs of the Hartley strain were actively sensitized with tubercle bacilli killed by heat and suspended in a Freund's type emulsion[4]. Spleen cells and lymph node cells were prepared as described previously[4]. Peripheral blood leucocytes were prepared by sedimenting the red cells from blood with 6 per cent dextran in saline and centrifuging the supernatant at 230 *g* for 15 min. The remaining contaminating red cells were then lysed in 0·05 *M* sodium chloride for 1 min. Preparations of peripheral leucocytes from individual animals contained 0·5–2·5 × 10^8 cells, while those from spleen and lymph nodes contained between 5 and 10 × 10^8 cells. Cell preparations were disrupted by freezing and thawing seven times in the presence of 0·1 mgm. deoxyribonuclease (Worthington Biochemical Corporation). Complete disruption of the cells was confirmed under the phase contrast microscope.

Live cell preparations were transfused intravenously, and disrupted cell preparations were injected intradermally into multiple sites on to one flank of the guinea pig. All skin tests were with 40 µgm. tuberculin (Mammalian *PPD*, from the Ministry of Agriculture's Veterinary Laboratory, Weybridge), which had been previously dialysed to remove the glycerol. Skin reactions were read 24 hr. after testing. Tests for anaphylaxis were with 2 mgm. *PPD* intravenously. Skin homografts from the donors of live cells were transplanted according to Billingham and Medawar[5].

The results of the transfusion of ~10^9 live cells from the spleen and/or lymph nodes of individual hypersensitive donors are shown in Table 1. The recipients were grafted with skin from the donors of the cells, and received their first skin test immediately after the transfusion. No correlation was observed between retention of skin graft and persistence of tuberculin sensitivity, and it is therefore very unlikely that the donor cells survived beyond one week. The one guinea pig which retained its skin graft for more than one month was found to have lost its tuberculin sensitivity by this time. Seven of the 13 guinea pigs maintained their sensitivity on repeated testing for more than 3 months, and 6 of these showed anaphylaxis when given 2 mgm. *PPD* intravenously at the end of this time.

Guinea pigs which had received intradermal injections of disrupted cells from hypersensitive donors were skin tested with *PPD* 5 or 6 times over a period of nearly 3 months, as shown in Table 2. Weak sensitivity to tuberculin developed 7–14 days later, in animals that had not had any previous injections of *PPD* and strong reactions were found on re-testing after 17 days. Half the animals showed strong reactions >11 × 11 mm. at some

Table 1. PERSISTENCE OF TUBERCULIN HYPERSENSITIVITY AFTER PASSIVE TRANSFER OF APPROXIMATELY 10^9 CELLS INTRAVENOUSLY

Day of skin test	Number of animals showing skin test reaction (40 µgm. *PPD*) after 24 hr.		Proportion rejecting skin graft	Anaphylaxis (2 mgm. *PPD* intravenous)
	Greater than 10 × 10 mm.	7 × 7–10 × 10 mm.		
0	6	7		
9 days	5	5	10/13	
1 month	4	2	12/13	
2 months	7	—		
3 months	7	—		6 (3 died, 3 mild)

Each of 13 guinea pigs received cells from one donor only and was grafted with skin from this animal.

Table 2. Development of Active Sensitization to Tuberculin in Guinea Pigs receiving Disrupted Cells from Peripheral Blood, Spleen, and Lymph Nodes of Hypersensitive Donors

		Days after injection of cells						
		0	7	13–14	17–25	33–43	61–65	82–85
	Peripheral leucocytes (~1 × 10⁸ cells)							
	No. tested	3	6	8	5	11	8	8
(a)	Strong reactions*	0	0	0	2	4	4	3
	Weak reactions	0	1	4	1	4	1	1
	Anaphylaxis†							7 (3 died)
	Spleen (~5 × 10⁸ cells)							
	No. tested	3	6	9	6	12	9	9
(b)	Strong reactions	0	0	0	0	0	2	4
	Weak reactions	0	1	4	3	7	1	3
	Anaphylaxis							8 (6 died)
	Lymph node cells (~5 × 10⁸ cells)							
	No. tested	—	—	6	3	6	3	3
(c)	Strong reactions	—	—	0	1	1	1	1
	Weak reactions	—	—	4	0	2	0	1
	Anaphylaxis							2 (1 died)
	No cells							
	No. tested	9	9	—	9	—	9	9
(d)	Strong reactions	0	0	—	0	—	0	0
	Weak reactions	0	1	—	0	—	0	2
	Anaphylaxis							2 (none died)

* Strong reactions were >10 × 10 mm.; weak reactions 7 × 7 to 10 × 10 mm. † Tested with 2 mgm. *PPD* intravenous.

stage or other and only 2 out of the 18 animals, which were followed over the whole period, failed to show any reaction. As a control, 9 normal animals were skin tested with *PPD* 5 times over a similar period. One of these showed reactivity on the second injection 7 days after the first, and afterwards failed to react, and 2 others showed very mild reactions which appeared only at the end of the three months course. When frozen and thawed peripheral blood leucocytes and spleen cells from normal unsensitized animals were used the recipients behaved like the control group with no cells.

At the end of the experiment all four groups were challenged with 2 mgm. *PPD* intravenously. Sixteen out of 18 animals which had received disrupted cells from hypersensitive donors developed anaphylaxis and 10 died. Only 2 of the 9 controls developed very mild anaphylaxis.

From these results it appears that a transient delayed hypersensitivity is transferred by intact peripheral blood leucocytes, spleen cells and lymph node cells, and that it may be followed by a superimposed active immunization which persists far beyond the time when the donor cells would have been destroyed by a homograft reaction. Peripheral blood leucocytes were more effective in respect of active sensitization than cells from the spleen and lymph nodes. A further property of cells from sensitized donors is that they can enhance the development of antibody against tuberculin accompanying multiple injections of *PPD*. *PPD* is not a single substance, and there is no evidence whether the antibody is directed against traces of contaminating antigen which are known to be present.

These experiments indicate that the possibility that active immunization has occurred must be borne in mind in the interpretation of experiments on passive transfer of delayed hypersensitivity.

I would like to thank Dr. J. H. Humphrey for his advice throughout this investigation.

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ENZYMATIC SYNTHESIS OF POLY-β-HYDROXYBUTYRIC ACID IN BACTERIA

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THE polymeric ester, poly-β-hydroxybutyric acid, originally discovered by Lemoigne in 1927 as a major component of *Bacillus megaterium*, has since been found to be an important storage material in a variety of bacteria[1–4]. It serves as an intracellular reserve of carbon and reducing power in the photosynthetic bacterium, *Rhodospirillum rubrum*, and as the principal substrate for the endogenous respiration of certain aerobes[3–5].

The present report deals with the synthesis of the polymer from carbon-14-labelled D(−)-β-hydroxybutyryl-coenzyme *A* by particulate fractions of cell-free extracts from *B. megaterium* and *R. rubrum*.

Radioactive D(−)-β-hydroxybutyric acid was obtained by alkaline hydrolysis of purified polymer isolated from cells of *R. rubrum* that had assimilated uniformly labelled acetate in the light[3]. The acid was purified by paper chromatography, and the coenzyme *A* derivative was prepared by the mixed anhydride method of Wieland and Rueff[6]. Free β-hydroxybutyric acid was removed from the acidified solution by ether extraction. Analysis of the specific

Table 1. SYNTHESIS OF POLYMER BY *B. megaterium* EXTRACTS

Time (min.)	Percentage carbon-14 recovered in polymer fraction		
	Exp. 1	Exp. 2	Exp. 3
0	0	0	0
5	—	27	—
10	—	35	—
20	33	45	46
40	36	44	—

The reaction mixtures contained either recombined particulate and supernatant fractions (1·08 mgm. polymer and 1·75 mgm. protein per ml.) or resuspended particulate fraction alone (1·08 mgm. polymer and 0·13 mgm. protein per ml.); and the following components (in μmoles per ml.): magnesium chloride, 12·5; glutathione, 12·5; and potassium phosphate buffer, *p*H 7·4, 125.

Exp. 1: particulate + supernatant fractions + 0·48 μequiv. D(−)-β-hydroxybutyryl-coenzyme *A* per ml. (initial); Exps. 2 and 3 particulate fraction + 0·24 and 0·48 μequiv. D(−)-β-hydroxybutyryl-coenzyme *A* per ml. (initial), respectively.

The reaction mixtures were incubated at 33° C.

activity of the product, coupled with hydroxamate and sulphydryl determinations, indicated that at least 95 per cent of the acid was present in thioester combination. Since the commercially available coenzyme *A* used for the synthesis is only 50–75 per cent pure and is contaminated with other sulphydryl compounds (for example, glutathione), the assays reflect the total concentration of β-hydroxybutyryl thioesters, rather than of the coenzyme *A* derivative alone. Poly-β-hydroxybutyric acid was assayed with a modified hydroxamate method for ester determination[7], or by the spectrophotometric method of Slepecky and Law[8].

B. megaterium, strain Km, was grown in the medium containing 2 per cent glucose described by Macrae and Wilkinson[4]. The cells were gathered at the end of exponential growth and washed with 0·033 *M* phosphate buffer, *p*H 7·0. Cell-free extracts were prepared by the osmotic lysis of concentrated protoplasts made by lysozyme treatment of the cells[9]. The lysates were subjected to sonic oscillation to liberate the polymer particles from the cell membranes. The heavy particulate fraction (consisting mainly of polymer particles) was separated from the extract by centrifugation at 3,000 *g* for 20 min. at 4° C., and the particles were washed several times with phosphate buffer, *p*H 7·0. The supernatant fraction contained both the soluble proteins of the cell and the disintegrated cell membranes.

The particulate fraction was incubated with radioactive β-hydroxybutyryl-coenzyme *A* in the presence and absence of the supernatant fraction. The reaction was stopped by addition of alcohol (60 per cent final concentration) and the radioactivity of the isolated polymer was measured. The particles were sedimented by centrifugation, treated with alkaline hypochlorite, washed successively with water and acetone and finally dissolved in chloroform[3,4]. Suitable aliquots were analysed for radioactivity. Controls were performed with reaction mixtures in which β-hydroxybutyryl-coenzyme *A* was replaced by labelled β-hydroxybutyrate in the presence or absence of coenzyme *A*, as well as by alkali-hydrolysed coenzyme *A* derivative. No incorporation of radioactivity into polymer was observed in the control reaction mixtures. In the presence of β-hydroxybutyryl-coenzyme *A*, on the other hand, the polymer became rapidly labelled with carbon-14 when the particulate fraction was used either alone or in combination with the supernatant fraction (Table 1).

The reaction came to completion in about 20 min., at which time 35–45 per cent of the total radioactivity was recovered in the polymer. The observed maximal conversion of thioester to polymer does not give any information on the equilibrium constant for the synthesis of polymer from β-hydroxybutyryl-coenzyme *A*, since the true concentration of the latter compound was not known for reasons previously discussed.

To study the stoichiometry of the reaction, a further experiment was performed in which the disappearance of thioester was measured. The analyses showed that in a reaction mixture initially containing 1·2 mgm. washed polymer particles (without supernatant fraction) and 1·2 μequiv. of thioester per ml., 0·5 μequiv. of β-hydroxybutyrate was incorporated in the polymer, while 0·6 μequiv. of thioester had disappeared. Thus, the synthesis of polymer accounted for more than 80 per cent of the thioester decomposed.

To identify the product unequivocally, the labelled polymer from the above experiment was diluted with purified carrier polymer[3,4] and reprecipitated three times from chloroform: twice with 95 per cent ethanol and once with acetone–ether (2 : 1). The specific activity remained constant throughout. The reprecipitated polymer was hydrolysed for 22 hr. at 30° in *N* potassium hydroxide, treated with cation exchange resin ('Dowex 50', H^+), and chromatographed on paper using the following solvents: (1) ether–benzene–formic acid–water (50 : 50 : 12·5 : 2); (2) *n*-butanol–glacial acetic acid–water (100 : 6 : 25); (3) *n*-butanol saturated with 1·5 *M* ammonia. In each case, only one radioactive spot was found on the paper, which corresponded exactly with the location of the β-hydroxybutyric acid spot.

Cell-free extracts of *R. rubrum* also rapidly incorporate the β-hydroxybutyryl moiety of the coenzyme *A* derivative into polymer. As in *B. megaterium*, the activity is largely associated with the particulate fraction. The kinetics of incorporation are, however, complicated by the presence of an active depolymerase which is also associated with the particles. Crude extracts were prepared by sonic oscillation of cells grown anaerobically in the light. The particulate and supernatant fractions were separated by low-speed centrifuging. The former, which contained the polymer particles, was heavily contaminated with pigmented cell debris. The native polymer, when resuspended in either the supernatant fraction or buffer, underwent rapid hydrolysis to β-hydroxybutyric acid. As much as 80 per cent of the polymer disappeared after 30 min. of incubation at 33° C.

The results of an experiment in which labelled β-hydroxybutyryl-coenzyme *A* was added to a crude cell-free preparation are shown in Fig. 1. Maximal incorporation was achieved within 2 min., after which time the total carbon-14 content of the polymer rapidly decreased. The early termination of incorporation can be plausibly ascribed to exhaustion of the substrate. Thereafter, radioactivity is lost from the polymer as a consequence of the continued depolymerization. The results of a parallel experiment, in which the resuspended particulate fraction was incubated with different concentrations of substrate, are entirely compatible with this interpretation (Table 2). The identification of the carbon-14-labelled product was carried out as described above in the experiments with *B. megaterium*.

No direct activation of β-hydroxybutyric acid with adenosine triphosphate and coenzyme *A* could be demonstrated in cell-free extracts of *R. rubrum*. A small incorporation of carbon-14 into polymer was

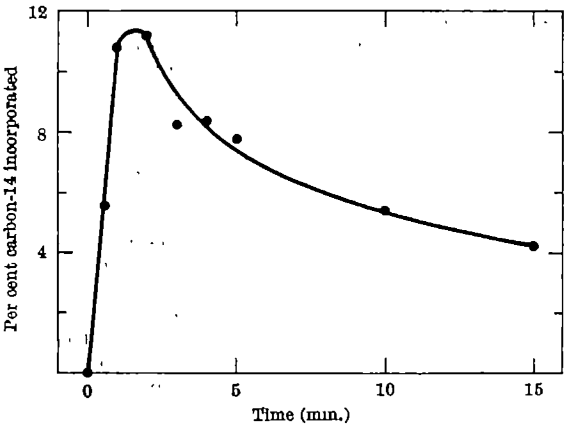

Fig. 1. Incorporation of carbon-14 into polymer by crude extracts of *R. rubrum*. The reaction mixtures contained crude extract (3·9 mgm. polymer and 5·08 mgm. protein per ml.); D(—)-β-hydroxybutyryl-coenzyme *A*, 1·4 μequiv.; and the following components (in μmoles per ml.): magnesium chloride, 12·7; glutathione, 12·7; and *tris* (hydroxymethyl) aminomethanehydrochloride buffer, *p*H 7·4, 127. The reaction mixtures were incubated at 33° C.

observed, however, when crude extracts were incubated with carbon-14-labelled acetate, adenosine triphosphate, coenzyme *A* and reduced pyridine nucleotides.

Two soluble enzymes, presumably involved in polymer metabolism, were found in the supernatant fraction of *R. rubrum* extracts prepared from cells that had depleted their polymer stores. A hydrolytic system catalyses digestion of boiled polymer particles of *R. rubrum* and native polymer particles of *B. megaterium* but does not attack the purified polymer. A specific diphosphopyridine nucleotide-linked D(—)-β-hydroxybutyric acid dehydrogenase is also present in such extracts, and has been partially purified.

Table 2. SYNTHESIS OF POLYMER BY PARTICULATE FRACTION OF *R. rubrum* EXTRACTS

Time (min.)	Percentage carbon-14 recovered in polymer fraction		
	Exp. 1	Exp. 2	Exp. 3
2	9	10	7
5	6	9	8
10	5	7	11

The reaction mixtures contained resuspended particulate fraction (3·1 mgm. polymer per ml.); and the following components (in μmoles per ml.): magnesium chloride, 10; glutathione, 10; and *tris* (hydroxymethyl) aminomethanehydrochloride buffer, *p*H 7·4, 100. Exps. 1, 2 and 3 contained 0·25, 1·0 and 4·0 μequiv. of D(—)-β-hydroxybutyryl-coenzyme *A* per ml. (initial), respectively. The reaction mixtures were incubated at 33° C.

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INHIBITION OF CELLULAR RESPIRATION BY CO-CARCINOGENIC FRACTIONS OF CROTON OIL

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As a result of the work of Rous and his colleagues[1] and Berenblum and his colleagues[2], it is widely accepted that carcinogenesis in skin—and probably also in other organs—takes place in two successive stages. A single small dose of a carcinogen such as 9 : 10-dimethyl-1 : 2-benzanthracene applied to mouse skin is sufficient to produce a lasting 'initiation' but no tumours. Subsequent repeated application of croton oil gives rise to a large crop of tumours, whereas croton oil applied without a previous initiating stimulus produces few, if any, tumours. Several arguments[3] make it clear that the 'promotion' or 'co-carcinogenesis' by croton oil is complementary to initiation and quite distinct from it; thus, administration of the initiator after prolonged treatment with croton oil does not lead to tumour formation.

The factor or factors in croton oil responsible for tumour promotion have not been identified, and their effects on cell metabolism have not previously been described. Lijinsky[4] has submitted methanolic extracts of croton seeds to silica-gel chromatography and obtained fractions, comprising about 1 per cent of the whole oil, that appear to have all its tumour-promoting activity. Fractions kindly provided by Dr. Lijinsky are at present being analysed by gas chromatography in this Institute in attempts to identify the active agent or agents. In the meantime, the opportunity has been taken—for reasons which will be apparent in the discussion here—to analyse the effects of the croton oil fractions on cell metabolism. It has been found that the highly active tumour-promoting fractions are inhibitors of cellular respiration in several tissues, and this property may well be related to their co-carcinogenic effect.

Rat liver homogenates. Livers of 100-gm. hooded rats were homogenized by the Potter–Elvehjem technique in the following medium: 0·3 *M* sucrose, 0·002 *M* sodium ethylenediamine tetraacetate and 0·03 *M* nicotinamide, adjusted to *p*H 7·1 with potassium hydroxide. Each Warburg flask contained 0·3 ml. homogenate (containing 1·02 mgm. protein nitrogen per ml.), 0·2 ml. potassium succinate, 0·15 *M*, *p*H 7·1 and 1·4 ml. sucrose–ethylenediamine tetraacetate–nicotinamide as above. Croton oil fractions were added in ten-fold dilutions in 0·05 ml. ethanolic solution to give final concentrations from 0·2 to 200 μgm. per ml. Oxygen uptake from air

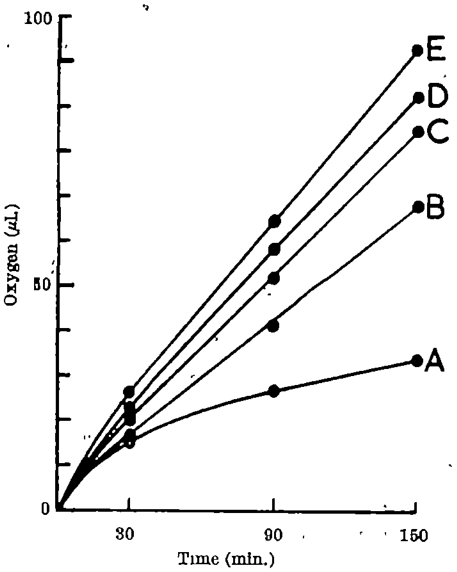

Fig. 1. Inhibition of oxygen consumption of rat liver homogenate with succinate as substrate by croton oil factor in various concentrations: *A*, 200 µgm. per ml.; *B*, 20 µgm. per ml.; *C*, 2 µgm. per ml.; *D*, 0·2 µgm. per ml.; *E*, no inhibitor

was measured in a Warburg apparatus for 2½ hr. at 30°. The results are shown in Fig. 1. Increasing concentrations of croton oil factor caused a progressive inhibition of respiration, up to 70 per cent inhibition at 200 µgm. per ml.

Rat liver mitochondria. Mitochondria were prepared from rat liver homogenates by five sedimentations and resuspensions in sucrose–ethylenediamine tetraacetate–nicotinamide medium. Each flask contained 0·3 ml. mitochondria (containing 0·33 mgm. protein nitrogen per ml.), 0·2 ml. sucrose–ethylenediamine tetraacetate–nicotinamide, 1·0 ml. reaction mixture and 0·1 ml. ethanol + croton oil factor. A reaction mixture devised for prolonged oxidative phosphorylation in rat liver mitochondria was used, giving final concentrations per ml. of: 100 µmol. sucrose, 40 µmol. potassium chloride, 20 µmol. nicotinamide, 1·3 µmol. ethylenediamine tetraacetate, 10 µmol. potassium succinate, 20 µmol. potassium phosphate, 4 µmol. adenosine monophosphate, 0·5 µmol. diphosphopyridine nucleotide, 20 µmol. glucose, 4·6 k*M* units hexokinase and 8 µmol. magnesium sulphate, *p*H 7·1. Oxygen uptake from air at 30° measured in the Warburg apparatus is shown in Fig. 2. The croton oil factor at a final concentration of 100 µgm. per ml. inhibited respiration by 70 per cent.

Mouse skin. Since most of the experiments on co-carcinogenesis by the croton oil factor have been carried out in mouse skin, the effect of the factor on respiration of mouse ear tissues—the metabolism of which is due mainly to skin—was investigated. Four young adult Swiss mice were used. To one ear of each mouse 50 µgm. of croton oil factor in benzene was applied, and to the other ears benzene alone. Four hours later, the animals were killed by dislocation of cervical vertebræ. The ears were cut up into small pieces about 1 mm. square. The pieces from the control ears were placed in two Warburg flasks containing 2·5 ml. Earle's solution with *M*/30 *tris*-(hydroxymethyl)aminomethane buffer, *p*H 7·4, and 0·05 ml. ethanol. The pieces from the ears treated with the croton oil factor were similarly prepared, except that the ethanol contained a solution of the factor, the final concentration of which was 100 µgm. per ml. Oxygen uptake from air was measured in a Warburg apparatus for 2½ hr. at 30°. After the experiment, excess moisture was removed from the tissues by blotting with filter paper; the tissues were weighed, and oxygen uptake corrected for weight. In both flasks containing the croton oil factor oxygen consumption was less than in control flasks, the inhibition being 46 and 52 per cent. These results suggest that respiration in skin, as in other tissues, is inhibited by the croton oil factor.

Heart muscle succinoxidase preparation. The activity of croton oil fractions on a heart muscle sarcosome preparation was also investigated. The method of preparation of material, spectroscopic observation of cytochrome oxidation and reduction and estimation of succinic dehydrogenase using the Thunberg technique were as previously described[6]. Measurements of oxygen utilization in the determination of succinoxidase and cytochrome oxidase activities were made polarographically with a continually cleaned silver electrode in a 3-ml. chamber completely filled to exclude air[7]. Succinoxidase activity was measured in the presence of *M*/50 sodium succinate in 0·14 *M* potassium phosphate buffer, *p*H 7·3. Cytochrome oxidase was measured in the presence of 0·05 *M* *p*-phenylenediamine and 0·14 *M* potassium phosphate buffer, *p*H 7·3. No additional cytochrome *c* was added, and all experiments were carried out at 30°. The final concentration of the heart muscle preparation used was 2·4 mgm. per ml.

Visual observation of the cytochrome absorption bands showed that the croton oil factor at a concentration of 100 µgm. per ml. inhibited the reduction of cytochromes *a* and *c* and the oxidation of cytochrome *b*. The inhibitory effect of various concentrations of the croton oil factor on overall succinoxidase activity is shown in Fig. 3. At a concentration of 50 µgm. per ml. there was also some inhibition of cyto-

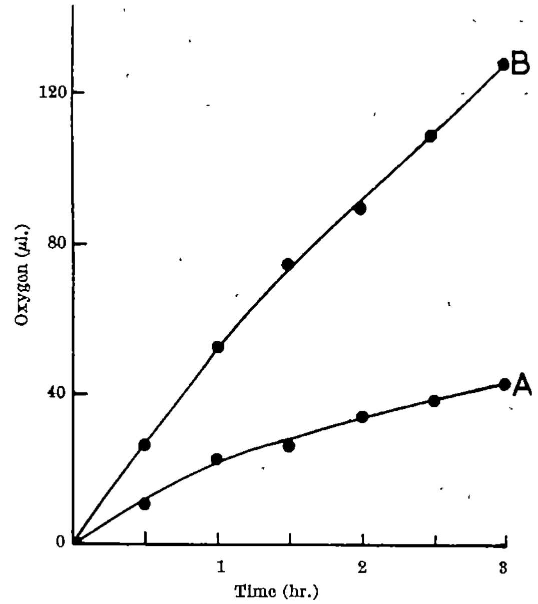

Fig. 2. Inhibition of oxygen consumption of rat liver mitochondria with succinate as substrate by croton oil factor. *A*, inhibitor at a concentration of 100 µgm. per ml.; *B*, no inhibitor

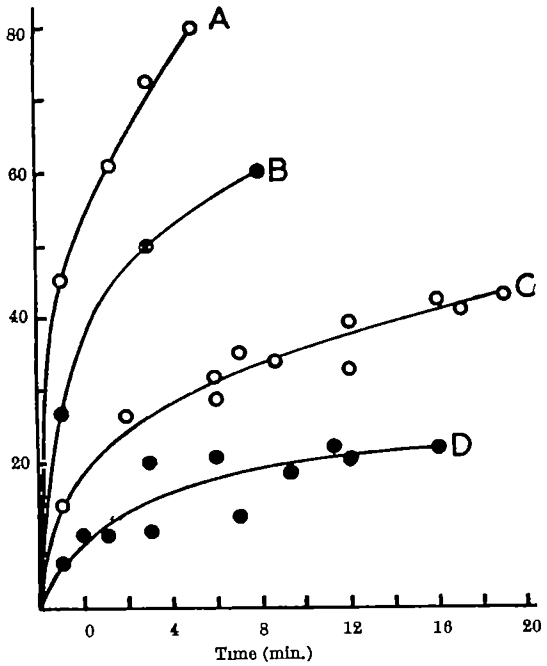

Fig. 3. Inhibition of heart muscle succinoxidase preparation by croton oil factor in various concentrations : *A*, 150 μgm. per ml. ; *B*, 100 μgm. per ml. ; *C*, 50 μgm. per ml. ; *D*, inhibition of cytochrome oxidase by croton oil factor at 50 μgm. per ml.

chrome oxidase, but no inhibition of succinic dehydrogenase. These observations show that the croton oil fraction used has several points of resemblance with antimycin *A* (ref. 8) and quinoline-N-oxide[9] in its metabolic effects. This pattern of inhibition has been shown to indicate a block in electron transport between cytochromes *b* and *c*. The inhibitory effect of the croton oil factor, however, developed more slowly than that produced by antimycin *A* and quinoline-N-oxide, and only the croton oil is also slightly inhibitory of cytochrome oxidase.

In general, the observations we have made suggest that the croton oil factor produces an overall inhibition of terminal respiration mediated through cytochromes. It seems unlikely that the effects reported here are due to alteration of the structural integrity of the cellular particles concerned. Phase-contrast microscopy of rat liver mitochondria exposed to 100 μgm. per ml. of the croton oil factor showed no detectable abnormality. At concentrations of the factor producing marked inhibition of overall succinoxidase activity there was much less inhibition of cytochrome oxidase and no detectable inhibition of succinic dehydrogenase activity.

The results described here show that fractions of croton oil having powerful co-carcinogenic activity contain inhibitors of terminal cellular respiration mediated through cytochromes that are effective in several tissues. It has been found that 100–200 μgm. per ml. of the croton oil fractions produce 70–80 per cent inhibition of respiration in liver and heart muscle preparations. When it is recalled that these fractions are still impure, and that owing to their lipid solubility penetration into tissues in aqueous suspension is poor, it seems likely that the factor or factors inhibiting respiration are highly active. They certainly produce detectable respiratory inhibition in skin at concentrations used for tumour promotion.

The only other metabolic effects of purified croton oil fractions so far described concern active transport of glucose by the isolated surviving guinea pig intestine[9]. The croton oil fractions were found to depress absorption of glucose under aerobic conditions to that observed under anaerobic conditions ; absorption under anaerobic conditions was not affected by croton oil, and other oils were without effect. An interpretation of these results consistent with our own findings is that croton oil depressed the yield from oxidative phosphorylation of adenosine triphosphate required for active transport of glucose. The yield of adenosine triphosphate from glycolysis was not apparently diminished by croton oil, which confirms our impression that the main inhibition is on terminal respiration, with little, if any, effect on other enzyme systems.

It is not yet established that the two properties of croton oil fractions, namely, respiratory inhibition and co-carcinogenesis, are related, but available evidence suggests that they may be. It is well known that tumour cells have high rates of aerobic as well as anaerobic glycolysis[10–12]; tumour cells are also able to use very efficiently adenosine triphosphate derived from glycolysis for protein synthesis[13]. It might therefore be expected that respiratory inhibition would retard growth and division of tumour cells less than that of normal, differentiated cells. In the mouse skin system which has been used for most work on co-carcinogenesis, this supposition seems to be well founded. There is substantial evidence that energy from oxidative phosphorylation is required for division of normal mouse skin cells[14], whereas preliminary results suggest that mouse skin papilloma cells continue to divide when cultured in an atmosphere of nitrogen and carbon dioxide in the presence of adequate glucose (Allison, A. C., unpublished work).

Promotion of co-carcinogenesis can therefore be envisaged as a process by which small numbers of tumour cells (produced by the initiator or carcinogen) are placed at a competitive advantage over normal cells the growth of which is handicapped by respiratory inhibition. Under the continued influence of the promoter, then, it can be supposed that tumour cells can form clones sufficiently large to become self-perpetuating. Experiments with other respiratory inhibitors are in progress to test this hypothesis further.

It should be noted that this hypothesis of carcinogenesis is the opposite of that proposed by Warburg[10]. According to Warburg, cancer cells are formed in two distinct stages : an irreversible lesion of cell respiration comes first ; as a result, some cells die but others become capable of replacing respiration by glycolysis as the main energy-producing reaction and are changed into cancer cells. However, careful search has failed to show substantial impairment of oxidative phosphorylation in tumour cells and mitochondria, and the general conclusion, discussed in detail by Weinhouse[11] and by Schlief and Schmidt[12], is that the high aerobic glycolysis of cancer cells is much less the result of a lesion in respiration than of an enhancement of glycolysis. The evidence thus supports our view that an early change in tumour cells is increased glycolysis, releasing the cells from strict dependence on energy derived from oxidative phosphorylation for growth. The phase of respiratory inhibition does not act directly on tumour cells, as Warburg supposed, but acts as a selective inhibitor of normal cells.

An attractive feature of this hypothesis of co-carcinogenesis is that it not only embraces the effects of anoxia from various causes but can also be extended to include co-carcinogenesis by viruses. Andrewes *et al.*[15] reported that when the fibroma virus—which normally produces benign, regressing lesions—was given to rabbits injected subcutaneously with tar, sarcomata were formed; no tumours were obtained with tar alone. Duran-Reynals[16] produced epitheliomata in the skins of fowls infected with fowl-pox virus and treated with sub-carcinogenic doses of methylcholanthrene; and Rous *et al.*[17] have shown that when the papilloma virus is given to rabbits, the skins of which had been treated with tar, malignant tumours develop rapidly. The nature and timing of the responses in these experiments indicate that the viruses (one of which, fowl-pox, is not a tumour virus at all) are acting as co-carcinogens, their effects being complementary to those of the carcinogens, tar and methylcholanthrene.

A common metabolic pattern in virus-infected cells is increased glycolysis[18], and, if there is competition for phosphate acceptors within the cells as envisaged by Chance and others[19], this would result in respiratory inhibition. Some of the metabolic effects in virus-infected tissues are probably due to interferon, which has been shown to increase glycolysis when added to cells[20]. Indeed, Isaacs[21] has already suggested that interferon may produce conditions favourable for the growth of tumour cells, and so act as a promoter. Increased aerobic glycolysis is a prominent feature of cells infected with the Rous sarcoma virus[22] and the mouse polyoma virus (Allison, A.C., unpublished work), so there is little doubt that these viruses can act as powerful promoters. Whether the tumour viruses—perhaps in contrast to other viruses—can also act as initiators remains to be determined.

We are indebted to Dr. W. Lijinsky for supplying purified croton oil fractions, to Dr. D. B. Roodyn for co-operation in some experiments and to Dr. A. Isaacs for helpful discussion.

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MORPHOLOGICAL CHARACTERIZATION OF THE VIRUS OF THE HUMAN COMMON WART (*VERRUCA VULGARIS*)

By M. G. WILLIAMS, A. F. HOWATSON and J. D. ALMEIDA

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IT is now more than ten years since Strauss *et al.*[1,2] published electron micrographs showing arrays of particles of average diameter 52 mμ in shadowed preparations of extracts obtained from human warts. These particles were believed to be the causative agent of certain types of human warts. Further evidence for the presence of specific particles in human wart tissue was obtained shortly afterwards by Bunting *et al.*[3], who were able to demonstrate arrays of particles within the nuclei of epidermal cells by using the newly developed thin-sectioning technique. The diameters of the particles seen in sections were in the range 20–38 mμ. The smaller size in sections was attributed to differences in preparative procedures. Although techniques for the morphological characterization of viruses have improved considerably since these investigations were carried out, little or no new information about the human wart virus has apparently been obtained. In a recent article, however, Siegel[4] has questioned the conclusion of Strauss *et al.* that the 52-mμ particles represent the human wart virus and has suggested that particles of diameter 16 mμ seen in his shadowed preparations are the causative agent.

In the present article, we describe observations on thin sections of human wart tissue, and on extracts of warts prepared for electron microscopy by the negative staining and shadow-casting techniques. By all three methods numerous particles of well-defined morphology were demonstrated, the most detailed characterization of the particles being obtained by the negative staining method.

Four common warts clinically typical of *verruca plana* and *verruca vulgaris* were examined by the thin-sectioning method. In three of these, the nuclei of some cells in the granular layer, close to the keratin layer, contained uniform particles of mean diameter 46 mμ. Most of the particles consisted of a dense core of diameter 34 mμ surrounded by a less-dense layer of width about 6 mμ, but in some the central region was less dense than the periphery. Fig. 1 illustrates the appearance of a typical group of particles. In one specimen the particles were for the most part arranged in close-packed crystalline formations; but in the others they were scattered at random throughout the nucleus. No particles were seen in the cytoplasm of any of the cells.

Two extracts were prepared, one from eight warts removed from several patients, the other from two warts obtained from two different patients. The warts were ground in a mortar with sand and distilled water and the homogenate clarified by

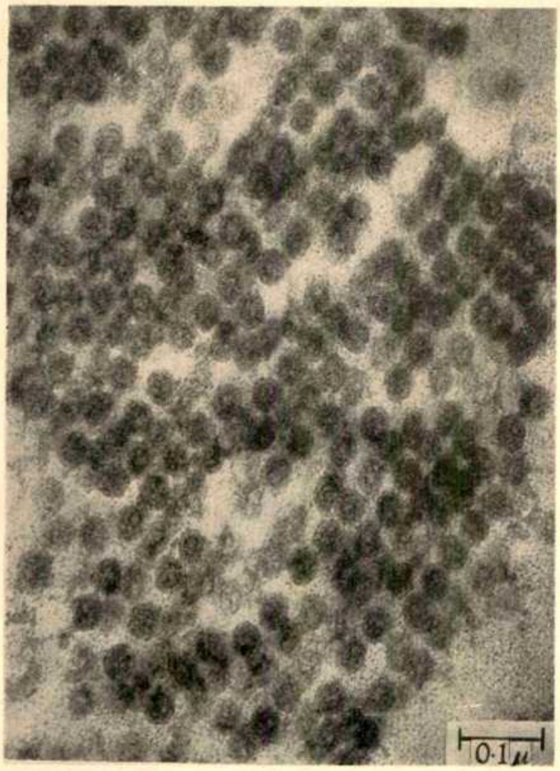

Fig. 1. Thin section showing part of an intranuclear aggregate of virus particles in a cell from the granular layer of a human wart

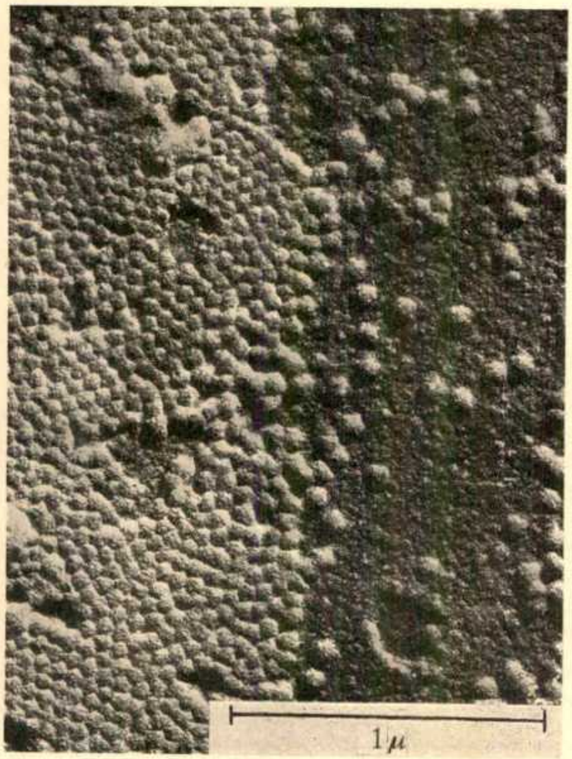

Fig. 2. Shadowed preparation from extract of human warts showing particles both scattered and in close-packed array

low-speed centrifugation. The supernatant was then centrifuged at 35,000 *g* for 2 hr., and the sediment from this centrifugation, re-suspended in a small volume of distilled water, formed the extract. Samples of extracts from both pools of warts were prepared for examination in the electron microscope by standard shadowing techniques, using gold-palladium alloy, and also by the negative staining method[5], using phosphotungstic acid brought to a *p*H of 5·4 with sodium hydroxide.

In shadowed preparations most of the specimen material present on the grid was in the form of particles similar in size and appearance to those described by Strauss *et al.*[1,2]. Fig. 2 shows a typical field. The average diameter of particles in close packed array is 55 mμ. Isolated particles appear appreciably larger, most having a diameter in the range 65–75 mμ. We were unable to detect the 16-mμ particles described by Siegel[4] in our preparations.

Specimens prepared by the negative staining method provided convincing evidence that the particles present in large quantities in our preparations were, indeed, viruses. By this method, it is apparent that the surfaces of the particles are composed of symmetrically arranged sub-units similar to those that have been described for other viruses[6,7]. Fig. 3*a*

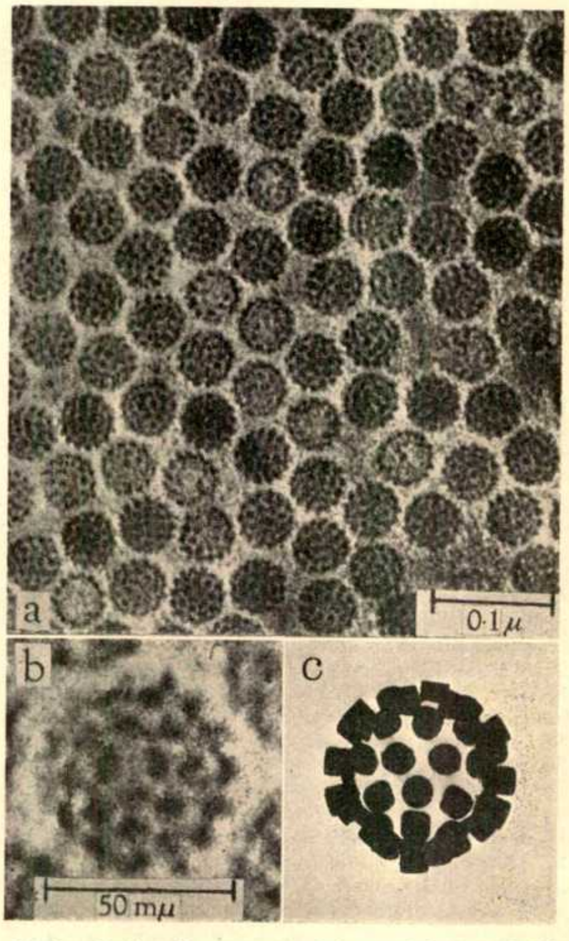

Fig. 3*a*. Portion of an aggregate of virus particles treated with phosphotungstic acid. Symmetrically arranged sub-units are visible on the surfaces of the particles; *b*, single virus particle at high magnification; *c*, model in same orientation as particle showing close correspondence in arrangement of sub-units

shows a small portion of a large aggregate of particles. In Fig. 3*a*, reproduced from a negative print, the sub-units appear dark against a light background. From observations of the arrangement of the sub-units or capsomeres on particles on which they are particularly distinct, it has been deduced that the shell or capsid of the particle consists of 42 capsomeres arranged in 5 : 3 : 2 axial symmetry. Twelve of the capsomeres are situated at the vertices of a regular icosahedron, and the remaining 30 are at the mid-points of the edges between adjacent triangular faces. The capsomeres have a diameter of approximately 8 mμ and are separated from one another by a distance of about 4 mμ. The overall diameter of the particles has an average value of 55 mμ. In Fig. 3*b* is shown an example of a particle in which virtually all the sub-units on one side of the particle are visible. Fig. 3*b* may be compared with Fig. 3*c*, which is a photograph of a model in the same orientation as the virus particle in Fig. 3*b*. In the negative-stained particle the peripheral capsomeres are more prominent than in the model, suggesting that the particle is somewhat flattened, but otherwise the correspondence between particle and model is close.

The number and arrangement of the capsomeres on the wart virus is the same as that established by Wildy *et al.*[7] for polyoma virus. The latter virus, however, is smaller, having a mean diameter of 45 mμ. It is of interest that the wart virus which produces a benign tumour in man is structurally similar to polyoma virus, which produces a wide spectrum of malignant tumours in several species of animals.

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HAPTOGLOBINS AND TRANSFERRINS OF TWO TROPICAL POPULATIONS

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THE haptoglobins are a family of serum proteins characterized by their ability to bind hæmoglobin. They have been studied extensively since 1938 by Jayle *et al.*[1]. Smithies[2] found that human populations could be divided into three types, termed 1–1, 2–2 and 2–1, dependent on their haptoglobin patterns. It has since been shown that for many populations the haptoglobins are determined by two autosomal genes, Hp^1 and Hp^2. Homozygous Hp^1 individuals are type 1–1; homozygous Hp^2 are type 2–2; and heterozygotes are type 2–1[3]. The genetics, and other features of this trait, have been reviewed recently[4,5].

A particularly interesting phenotype is that in which no hæmoglobin binding can be demonstrated. This type (designated *O*) is most common in West Africans[6], but it is also found in other populations[7-9]. There is, as yet, no adequate genetic explanation for this type. There is a striking difference in the distribution of the phenotypes in different populations[10]; and this is presumably due, in part, to differences in the selective forces which operate to maintain this genetic polymorphism. In this regard, it is interesting that there does not appear to be a polymorphism in some lower primates; a haptoglobin band which migrates with approximately the mobility of human haptoglobin type 1–1 was the only one found in a study of 170 *Macaca mulatta* monkeys, 4 chimpanzees, and 34 baboons[11]. Haptoglobin 2–1-like sera are, however, seen in some other species[11a].

Transferrin (or siderophilin) is the iron-binding protein of serum. Most American White individuals have a single iron-binding band, termed transferrin *C*. However, in a small number of American Negroes, and in a larger number of Australian Aborigines, a second band, transferrin *D*, is found; and a fast-moving band, termed *B*, is found in some European and Asian populations. Smithies and Hiller[12] have demonstrated that the transferrins are genetically controlled at a single locus *Tf* by at least three alleles, Tf^B, Tf^C and Tf^D. Transferrins of intermediate frequency, termed B_1, B_2, D_1, D_2, D_3, have also been found, but their genetics is not yet completely determined.

During the 1959 health survey of the Micronesian inhabitants of Rongelap Atoll, Marshall Islands (who had accidentally been exposed to radiation fall-out in 1954[13]) blood samples were collected for hæmatological and other studies. An aliquot of the serum was made available for the present examination.

Rongelap is in the Ralik (Western) chain of the northern Marshalls at approximately 11° N. The inhabitants are Micronesians, an Oceanic group, who have affinities with Polynesians and Melanesians, and, presumably, remotely with inhabitants of south-east Asia. Serum was obtained from 176 individuals, representing 70 per cent of the inhabitants at the time of the visit. Also studied were 118 sera collected in Rongelap in 1957, and kept frozen since their collection. The 1959 collection included nearly all the individuals sampled in 1957.

The West African sera were collected from Nomadic (or Pastoral) Fulani in several regions of northern Nigeria (at approximately 12° N.) in the summer of 1957. The population studied is described in greater detail elsewhere[14]. The red blood cells and serum were separated shortly after collection, and the latter frozen until the haptoglobin and β-globulin determinations were made in 1959. We are indebted to Dr. Hayhurst, medical officer at Pankshin, Nigeria, for supplying 59 of the sera. In all cases, blood was removed by venipuncture and allowed to clot in a dry test-tube at room temperature. The serum was separated by centrifuging and pipetting.

Table 1. DISTRIBUTION OF HAPTOGLOBIN AND TRANSFERRIN TYPES IN RONGELAP AND FULANI POPULATIONS

Population	Haptoglobins									Transferrins						
		1–1		2–2		2–1		0			*CC*		*CD*		*DD*	
	Total No.	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	Total No.	No.	Per cent	No.	Per cent	No.	Per cent
All Rongelap	176	59	33·5	31	17·6	83*	47·2	3	1·7	66‡	100	100	—	—	—	—
Rongelap, selected	124	41	33·1	18	14·5	62*	50·0	3	2·4	—	—	—	—	—	—	—
Fulani	84	24	28·6	4	4·8	14*	16·6	42†	50·0	68	57	83·8	10	14·7	1	1·5

* Includes one type 2–1 *M* ('type *F*' of Blumberg *et al.*) (ref. 7).
† Includes two sera in which only the fourth fastest band of type 2–1 was visible.
‡ In addition to the 66 sera studied by the *tris* discontinuous buffer method, 40 were studied by the horizontal borate method and found to be type *CC*.

We used the starch-gel electrophoresis method of Smithies[3], with borate buffer, and the conditions for serum described by him. Approximately 80 per cent of the sera, including all those of haptoglobin type *O*, were also run with the discontinuous (*tris*) buffer system of Poulik[15]. The haptoglobins were identified by adding human hæmoglobin solution to an approximate concentration of 120 mgm. per 100 ml. to serum which was not already hæmolysed, and staining for hæmoglobin with benzidine and hydrogen peroxide[16]. Transferrins were determined by the discontinuous buffer system on 68 Fulani sera and 66 Micronesian sera. In addition, observations on an additional 40 Micronesian sera were made using the borate buffer, horizontal method. No types other than *CC* were seen in the Micronesian sera. All sera which were of phenotypes other than *CC* were also studied by the vertical borate method of Smithies[17]. In confirming the transferrin types of sera other than type *CC*, the position of the protein was confirmed by adding to the serum radioactive ferric chloride (obtained from Abbott Laboratories, Oak Ridge, Tennessee) to a final concentration of approximately 3 × 10^{-5} molar iron, and subjecting the resulting mixture to electrophoresis in the usual manner. Radioautographs of one-half of the slit gel were made by the method described by Blumberg and Robbins[18].

The distribution of the haptoglobin types in the 176 Rongelapese tested is shown in Table 1. This sample includes some families in which two or more offspring are included. In these, all but one sibling were removed by random selection. This resulted in a sample of 124 individuals in which, at most, the parents and one child of a family were included. The distribution of the haptoglobin types in this group did not differ significantly from that in the entire group. In each case, agreement with the Hardy–Weinberg predictions was good, suggesting that the population was homogeneous for this trait. Omitting the three sera with no haptoglobins, the frequency of the Hp^1 gene is 0·58 and of the Hp^2 gene is 0·42. The frequency of the Hp^1 gene is higher than in European populations so far tested.

There were four Rongelapese who had no detectable haptoglobin, either in 1957 or 1959. In addition, there were many sera in which very small amounts only of haptoglobin were visible. This included two which were very faint 2–2's, but in which the fastest-moving haptoglobin band was not seen.

There were two sera (30, 830) which had no detectable haptoglobin, both in 1957 and 1959; in a third (933) only a 1959 serum was available, and this was ahaptoglobinæmic. One serum (842) was a very faint 2–2 in 1957, but ahaptoglobinæmic in 1959. Another (822) was a faint 2–2 in 1957, and in 1959 had a barely detectable amount of haptoglobin, which could not be typed. There was one individual (882) who had a pattern in which the fast-moving haptoglobin band of type 1–1, and the second fastest-moving haptoglobin band of a type 2–1 was present. This is similar to the 'type 2–1 *M*' described by Smithies and Connell[4], which appears to be identical with the 'type *F*' pattern of Blumberg *et al.*[7]. The pedigree of the family with the unusual types is shown in Fig. 1.

The Micronesian sera studied were all transferrin type *CC*. This includes 66 studied by the *tris* discontinuous buffer method and 40 by the horizontal borate buffer method.

The distribution of the haptoglobin types in the Fulani is shown in Table 1. More than half had no detectable haptoglobin, the highest frequency of this type yet reported. Included in the ahaptoglobinæmic sera were two in which only a single band corresponding to the fourth fastest moving band of type 2–1 was visible. This variant had not been seen in any previous studies. Of those who could be typed, nearly 60 per cent were type 1–1, and only four were type 2–2. 15 per cent of the 68 sera had both transferrin *C* and *D*, and one of them had only transferrin *D* (Table 1). Assuming that the genetic hypothesis of Smithies is correct, the distribution of the phenotypes is consistent with the Hardy-Weinberg prediction; and the frequency of the Tf^D gene is 0·1. The serum of the single *D* phenotype was used in studies on the chemical structure of the transferrin to be reported elsewhere[19]. All the slow-moving transferrins had

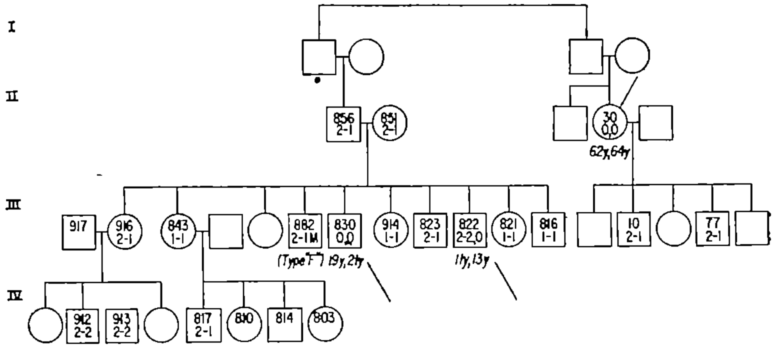

Fig. 1. Kinship of individuals with unusual haptoglobin types (Rongelap. Marshall Islands)

The results of the studies on the 1957 and 1959 sera are shown for those individuals with ahaptoglobinæmia identical mobility, and no fast-moving transferrins were found.

Considerable caution must be exercised in extrapolating to an entire population the gene frequencies obtained from a small sample. This is particularly true when studying societies made up of small isolated or semi-isolated groups in which genetic drift might be expected to operate. Thus, the Rongelapese appear to have a fairly high frequency of type 1–1. However, this may not be the case for all the Marshall Islands. Approximately 60 sera were obtained from each of two other Marshall Islands, Majuro and Utirik. In each of these the frequency of type 1–1 was lower than in Rongelap and closer to the European frequency.

Most of the Fulani sera which could be typed were of type 1–1, and the frequency of the Hp^1 gene was very low indeed. This is consistent with other findings from West Africa[6,10], in which as many as 50 per cent of the samples were of type 1–1. The frequency of Fulani with no haptoglobin is, to our knowledge, the highest yet reported. Each of the *O* types was re-checked two or more times, using the *tris* buffer, and, in addition, approximately half of them were re-checked using the vertical gel method with borate buffer. Only those in which there was no benzidine staining material in the region of the haptoglobins were scored as type *O*.

It is clear from the Micronesian studies that, at least in some cases, an individual may have no haptoglobin at one point in time, but have sufficient haptoglobin to permit typing at another time. (In this case, the interval of sampling was two years.) A complete medical and physical examination and determination of the hæmoglobins, red blood counts, white blood counts, and hæmatocrit were performed on all the ahaptoglobinæmic individuals on Rongelap. No abnormalities which might differentiate them from individuals with typable haptoglobin were noted. Both the individuals, who at one time were typable and at another were ahaptoglobinæmic, were of phenotype 2–2. This was also true in the two cases reported by Galatius-Jensen[9]. However, Sutton *et al.*[10] reported a single individual who was at one time ahaptoglobinæmic but who could later be typed as a 2–1. A further case in point is a patient studied by Shulman and Blumberg[21]. A five-year-old schoolboy developed a hæmolytic anæmia due to the presence of Donath-Landsteiner cold hæmolysis, of unknown ætiology. There were two acute episodes of hæmolysis within 48 hr., but no further evidence of clinical hæmolysis after this. One week after the initial hæmolytic episode, there was no detectable haptoglobin in the boy's serum. One month later, 2 bands corresponding to the second and third fastest bands of type 2–2 were detected. These bound approximately 20 mgm. of hæmoglobin per 100 ml. of serum. Three months after the initial hæmolytic episode, the patient had a normal 2–2 pattern which bound 120 mgm. of hæmoglobin per 100 ml. of serum, approximately a normal amount. However, retesting the serum of the patient one year after the original hæmolysis again failed to detect any haptoglobin despite the fact that there had been no clinically detectable hæmolysis in the interim. Apparently the patient had been intermittently ahaptoglobinæmic and in one case without regard to the presence of the hæmolytic episode. The patient's mother was a type 2–2, but bound an abnormally low amount of hæmoglobin (approximately 20 mgm. of hæmoglobin per 100 ml. of serum).

The ahaptoglobinæmia kinship (Fig. 1) does not help to elucidate the genetics of this type. One of the sibs of an ahaptoglobinæmic was a type 2–1 *M*. Giblett[22] has reported that many ahaptoglobinæmic children were born to matings in which at least one parent had the phenotype 2–1 *M*.

An explanation of the ahaptoglobinæmic type is still not available. In some cases, at least, it appears to vary during life ; and it is known that most infants do not have haptoglobin. The familial clustering, and the association with the 2–1 *M* type, suggest that some inherited factor is involved. The most probable explanation is that there is an inherited propensity to develop ahaptoglobinæmia, and that under different environmental conditions, ahaptoglobinæmia may develop. Under extreme conditions, such as severe hæmolytic anæmia, any individual could develop ahaptoglobinæmia. It is known that other pathological conditions such as liver disease, uræmia and Addison's disease can also lead to a decrease in haptoglobins.

The relatively high frequency of the transferrin types *CD* and *DD* in the Fulani is consistent with the findings in other African groups[22]. On the basis of the small number studied, this appears to be among the highest frequencies found in Africa, and is second only to the prevalence in Australian Aborigines. The complete absence of transferrin *D* in the Micronesian sample is in interesting contrast to the findings on the Fulani. There is no evidence as to what forces maintain this polymorphism in some but not other populations.

We are grateful to Dr. R. A. Conard, leader of the Brookhaven National Laboratory medical survey team to Rongelap, for his assistance in securing the Micronesian blood samples and for his encouragement and help in the conduct of this work. Some of the Fulani blood samples were collected during an expedition to Nigeria supported in part by the Wenner-Gren Foundation, Inc. Mr. O. Oyefesu, Ibadan, assisted in the collection of the Fulani samples.

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MODE OF ACTION OF AN ANTHERIDIUM-INDUCING SUBSTANCE IN FERNS

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THE control of antheridium formation in certain fern species by a naturally occurring substance[1,2] has been reviewed before in detail[3]. The substance is specifically concerned with the formation of male sex organs in many species of the family Polypodiaceæ and the representative tested of the family Dicksoniaceæ, while the species investigated of some other fern families (Cyatheaceæ, Osmundaceæ, Schizaeaceæ) are unresponsive to it[4,5].

The prothalli of *Onoclea sensibilis* and *Pteridium aquilinum* form antheridia in response to the active substance only during an early phase of development. They become insensitive to it soon after they become heart-shaped, and a few days prior to attaining the archegonial phase. Once insensitive, the *Onoclea* prothalli fail to form antheridia even if supplied with the substance at a concentration 15,000 times as high as that sufficient to induce antheridia in prothalli just two days younger[6]. Soon after the prothalli of *P. aquilinum* and of many other polypodiaceous fern species attain the archegonial phase, the last of the ephemeral antheridia formed during the sensitive phase disintegrate.

Czaja[7] and Dopp[8] observed that meristemless fragments excised from the archegonium-bearing prothalli of *Blechnum brasiliense* and of *P. aquilinum*, respectively, proceeded to form antheridia again. Demeristemized, archegonium-bearing *Onoclea* prothalli which were cultured on medium supplemented with antheridial factor also gave rise to antheridia[3]. It has already been mentioned that the archegonium-bearing prothalli of this species are no longer responsive to the antheridial factor. The re-appearance of antheridia in the meristemless fragments shows that the loss of sensitivity to the active substance is maintained as a function of the meristematic region.

Further investigations which were carried out on wings (meristemless fragments), excised from 24-day-old (insensitive) prothalli and cultured on medium containing antheridial factor, showed that the lag period between excision and the first appearance of antheridium initials varied considerably, ranging from 7 to about 14 days[3]. In contrast, most intact prothalli responded to the antheridial factor within 2½–3½ days while they were still at the sensitive stage. Thus the wings excised from the insensitive prothalli formed antheridia with considerable delay. This delay cannot be ascribed to damage incurred by excision because regenerative cell division could be clearly discerned within one day in a few of the wings and within two days in a majority of them. The delay may be understood, however, if we assume that the restoration of sensitivity to the antheridial factor follows excision not abruptly but gradually. Further observations indicated that antheridia arose only in those regions of the regenerating wings that had first undergone a number of vegetative cell divisions[3]. Perhaps, therefore, sensitivity to the antheridial factor is restored as a function of regenerative cell division.

It could be further observed that many excised wings gave rise to antheridia even on medium not supplemented with the antheridium-inducing substance. This is quite surprising if we consider that intact prothalli of this species fail to form antheridia spontaneously under the prevailing conditions of culture[3].

This observation suggests the possibility that meristemless fragments give rise to antheridia without the intervention of the antheridial factor. In turn, such an assumption leads to the hypothesis that the antheridial factor acts by overcoming a block to antheridium formation that is imposed on the prothallus by the meristematic region.

The hypothesis implies that this block to antheridium formation is operative as soon as the prothallus responds to the antheridial factor, that is, at a very early stage of development. In contrast, the prothalli do not become insensitive to the antheridial factor until after they have attained heart shape. The postulated reversibility by the antheridial factor of the newly proposed block also contrasts with the finding that the loss of sensitivity cannot be reversed by that factor[6]. The two phenomena should therefore be treated as distinct events in the development of the *Onoclea* gametophyte.

The present report is concerned with an attempt to put the proposed hypothesis to an experimental test.

The procedures used in the sterilization and inoculation of the spores have been described elsewhere[4,6]. A detailed description of the conditions of culture has also been given. The prothalli were cultured in 50-ml. flasks which contained 10 ml. of the mineral medium solidified with 1 per cent agar. Antheridium-inducing activity was assayed against prothalli of *O. sensibilis* by means of a dilution series. The prothalli of this species proved ideal for this purpose because they failed to form antheridia spontaneously under the prevailing conditions of culture, but formed them readily if the medium was supplemented with antheridial factor[3]. The active substance was supplied as the diluted liquid medium from 7-week-old cultures of *P. aquilinum*, which was commonly active to a dilution of 1 : 30,000 (ref. 2).

With the proposed hypothesis in mind, the formation of antheridia by demeristemized prothalli was studied more closely. Two hundred wings (that is, meristemless parts of the gametophytes) were excised from 16-day-old *Onoclea* prothalli and transferred to new medium in 50-ml. flasks that was supplemented with antheridial factor (supplied as 1/100 full strength *Pteridium* medium that elicited an antheridial response to a dilution of 1 : 30,000). Another 200 wings were transferred to medium not supplemented with antheridial factor. Five wings were cultured in each 50-ml. flask. Samples of 20 regenerating fragments were then examined for the presence of antheridia at intervals of one

Table 1. FORMATION OF ANTHERIDIA BY EXCISED WINGS ON MEDIUM WITH AND WITHOUT ANTHERIDIAL FACTOR

Days after excision	Plus antheridial factor		Minus antheridial factor	
	No. of wings with antheridia	No. of wings with archegonia	No. of wings with antheridia	No. of wings with archegonia
4	0	0	0	0
5	0	0	0	0
6	1	1	0	0
7	5	1	3	1
8	7	2	5	3
9	9	3	8	4
11	11	4	10	6
13	14	8	11	8
15	15	10	9	12
17	13	14	7	17

day or, at later stages of regeneration, at intervals of two days. These observations were continued until the number of antheridium-bearing fragments began to decline. This decline occurs because the regenerating fragments stop forming antheridia when they attain the archegonial phase, as is the case with the prothalli of most polypodiaceous species.

Table 1 shows that the maximal counts of antheridium-bearing regenerates never reach twenty, that is, the number of wings examined at each interval. This indicates that some of the regenerating wings lack an antheridial phase. It seems possible, however, that the antheridia observed on the regenerating wings with an early male phase disintegrate before other regenerates attain this reproductive phase. Thus, the data of Table 1 seem equally consistent with the assumption that all the excised wings form antheridia.

Fifty wings that bore antheridia only 10 days after excision were returned to the medium and re-examined seven days thereafter. Thirty-eight of the fragments then bore 1–4 archegonia. All regenerates with one or two archegonium initials and three out of five wings with three such initials then still bore antheridia. In contrast, the lone fragment which had already given rise to the fourth archegonium lacked antheridia. Whereas these results pertain to wings cultured on medium supplemented with antheridial factor, it was demonstrated that on unsupplemented medium the last-formed antheridia also persist until at least two archegonia have been initiated. The regenerating *Onoclea* wings behave similarly in this respect to intact prothalli of *P. aquilinum*[4].

The wings which attained the archegonial phase up to 11 days following excision in the presence of antheridial factor, or up to 13 days following excision in its absence, invariably lacked antheridia even if they had initiated only the first archegonium. Bisexual wings first appeared among the 20 wings examined two days thereafter. (This is reflected in the fact that the sum of antheridium-bearing and of archegonium-bearing wings then surpasses 20 for the first time ; see Table 1.) Their number increased in the samples examined afterwards, and bisexual wings with up to three archegonial initials could then be seen. It may be concluded that among wings exposed to antheridial factor approximately four wings (that is, the number of wings that have reached the archegonial phase prior to the first appearance of bisexual wings ; see Table 1) to about five wings (that is, 20, the number of wings examined per interval, minus 15, the maximal count of antheridium-bearing wings) out of 20 failed to form antheridia. Based on these same considerations, it is inferred that, among wings not exposed to antheridial factor, about eight or nine out of 20 attained the archegonial phase without a prior antheridial phase. The available data thus permit the conclusion that the maximal counts of antheridium-bearing wings reflect accurately, or with little error, the proportion of wings with an antheridial phase.

As already stressed, the maximal count of antheridium-bearing wings is higher in the presence of antheridial factor (15 out of 20) than in its absence (11 out of 20). The difference was even more pronounced in three repetitions of this experiment. The conclusion was drawn that the addition of the antheridial factor increases the proportion of antheridium-forming regenerates. Table 1 further shows that the first antheridium-bearing fragment appears one day earlier in the presence of antheridial factor than in its absence. Results of the three repetitions are in agreement with the conclusion that antheridium-bearing wings can be seen earlier in the presence of the antheridial factor than in its absence.

Fragments regenerating in the presence of antheridial factor may give rise to antheridia in the interval following the decay of the loss of sensitivity imposed on the wing by the meristem of the mother prothallus and prior to its re-imposition by a newly regenerated meristem. Thus, the regenerates with an early archegonial phase (those with the most rapid rate of regenerative development) would seem to lack an antheridial phase despite the presence of the active substance because this interval is too brief or non-existent.

In the absence of the factor, the phase of regenerative development conducive to antheridium formation is, in the context of the hypothesis, expected to be still briefer if it is recalled that the postulated block to antheridium formation is operative before the loss of sensitivity sets in. It seems predictable, therefore, that, for antheridia to arise in the absence of the antheridial factor, the rate of regenerative development must fall to a still lower value than in the presence of that factor. This consideration may explain the lower proportion of antheridium-forming regenerates in the absence of that factor. It may also account for the delayed appearance of antheridium-bearing wings in the absence of antheridial factor if we consider that wings which first form antheridia in the presence of antheridial factor might undergo regenerative development at too rapid a rate for the formation of these sex organs on medium not supplemented with that factor.

The hypothesis that the excised wings form antheridia without the intervention of the antheridial factor could not remain unchallenged when it was found that the prothalli of *O. sensibilis* produce some antheridial factor even though they fail to form antheridia under the conditions of culture. In the medium, a trace of activity could be first detected at the highest concentration applied (1/2 full strength) 16 days after inoculation. A maximum was reached in approximately 30-day-old cultures when the medium was active to a dilution of 1/10, more rarely 1/30 full strength. The titre remained approximately constant thereafter for a considerable period of time. The 16-day-old prothalli are so small that it was not feasible to obtain a sufficient amount of extract to assay for activity. Extract from 24-day-old gametophytes was active to a dilution of 1/10, more rarely 1/30 strength. As reported earlier, the extract of older, 45-day-old, prothalli was mostly inactive even at 1/2 full strength[8], but some activity could then be regularly detected in the medium. Perhaps this finding relates to the observation that the basal region had become necrotic in many prothalli of that age.

It should be noted that the last individuals of the gametophyte population became insensitive to the antheridial factor 2–3 days before a trace of antheridium-inducing activity could first be detected in the medium. The results thus indicate that *O. sensibilis* fails to form antheridia under the prevailing conditions of culture because the antheridial factor does not become available until all prothalli have become insensitive to it.

The possibility should be weighed that the wings excised from the 16-day-old prothalli give rise to antheridia in response to the small amount of antheridial factor which they probably contain upon excision. Various experiments indicated, however, that the wings do not elaborate the active substance and that the small amount that may be present on excision would become equilibrated with the medium to an ineffective level before sensitivity to the antheridial factor is restored. Even though these results are consistent with the hypothesis that the excised wings give rise to antheridia without the intervention of the antheridial factor, they cannot be considered conclusive. For this reason no attempt is made to describe the experiments in detail.

It should be possible, however, to obtain more conclusive information with young (still sensitive) prothalli which, according to the available criteria, do not yet elaborate the antheridial factor. Attempts to excise non-meristematic regions from such tiny prothalli proved unsuccessful because most or all of the excised cells died. An alternative approach was therefore chosen.

It was demonstrated many years ago[9,10] that plasmolysis of fern prothalli leads to regenerative cell division and the formation of regenerative outgrowths. Plasmolysis is considered to break the cytoplasmic connexions between neighbouring cells. The authors of these papers concluded that the observed regenerative activity resulted from the disruption of cytoplasmic continuity and the ensuing breakdown in correlative interaction between the meristem and the peripheral areas of the gametophyte.

If the postulated block to antheridium formation actually exists as a function of the meristematic region, then the severance of the cytoplasmic connexions might lead to a decay of the postulated block to antheridium formation and, as a consequence, to the formation of antheridia on medium not supplemented with antheridial factor (that is, in the context of the hypothesis, without the intervention of that factor).

Prothalli on about 1½-mm. thick agar slivers were removed from 6-day-old cultures with a spatula and transferred to 30 ml. of the plasmolytic agent in a Petri dish. The agar slivers which were about 3/4 cm.^{2} in surface area were submerged by applying gentle pressure with the flat surface of the spatula, thus ensuring simultaneous onset of plasmolysis in all prothalli. Following an exposure of 35 min. to the plasmolytic agent, the prothalli, on their agar supports, were transferred to 30 ml. of liquid medium in a Petri dish, where they remained for 35 min. The deplasmolysed prothalli were then returned to agar medium, two agar slivers with prothalli per 50-ml. flask. Sucrose, calcium chloride and mannite were used as the plasmolytic agents.

The temporarily plasmolysed prothalli gave rise to antheridia. Each of the three compounds elicited an antheridial response, even at concentrations that brought about only slight plasmolysis (0·4 *M* sucrose or mannite; 0·15 *M* calcium chloride). The prothalli failed to form antheridia when they were treated with non-plasmolytic concentrations of the three substances (0·3 *M* sucrose or mannite; 0·1 *M* calcium chloride).

The demonstration that only plasmolytically active concentrations are effective supports the assumption that the observed antheridia arise as a function of plasmolysis. This postulate is further strengthened by the finding that the spectrum of effective compounds comprises such chemically diverse compounds as an inorganic salt, a metabolically active organic compound (sucrose) and an organic compound which in all likelihood is metabolically inert (mannite). In turn, it is likely, but difficult to demonstrate conclusively, that plasmolysis is effective because of the ensuing disruption of cytoplasmic continuity.

In a few of the prothalli, antheridium initials could first be seen three days after the 6-day-old individuals were plasmolysed (45 min., 0·6 *M* mannite). (An antheridium was considered to be initiated in the context of this investigation when it appeared as the characteristic semi-circular outgrowth on the initiating vegetative cell.) In non-plasmolysed prothalli the first appearance of antheridial initials followed exposure to antheridial factor within 2½ days in both 4- and 6-day-old prothalli and within a wide range of concentrations (1/10, 1/70 and 1/500 full-strength *Pteridium* medium that was active to a dilution of 1 : 6,000). It is possible, however, that slight differences will be found if the interval of observation used (1/2 day) is shortened. All prothalli ultimately formed antheridia at the above concentrations, while only one in about four responded if the concentration was further lowered to 1/2,000 full-strength *Pteridium* medium. At this limiting concentration, an initial was occasionally found again 2½ days after the antheridial factor was supplied if a large enough sample of prothalli were examined. In other instances three days elapsed at this concentration until antheridium initials could first be seen on a few individuals of the gametophyte population.

If the prothalli subjected to plasmolysis at the 6-day stage were provided with antheridial factor (1/100 full-strength *Pteridium* medium) immediately following deplasmolysis, then the lag period appeared to be intermediate between that encountered with plasmolysed prothalli not exposed to antheridial factor and that observed in non-plasmolysed prothalli exposed to the active substance, that is, about 2¾ days elapsed between deplasmolysis and the appearance of the first antheridium initials.

The above results show that the temporary plasmolysis of 6-day-old *Onoclea* prothalli leads to the first appearance of antheridium initials nine days after inoculation. These initials cannot be accounted for in terms of the antheridial factor unless the assumption is made that the factor is present in the 6½-day-old prothalli (nine days minus the interval of somewhat more than 2½ days between the exposure of the plasmolysed prothalli to antheridial factor and the first appearance of antheridium initials).

If the antheridial factor were available to 6½-day-old prothalli in the normal sequence of events, then at least some of the individuals in a population of unplasmolysed gametophytes should give rise to antheridia because all are then sensitive to it (with the spore sample used in these experiments, the first individuals of the gametophyte population became insensitive eight days following inoculation). As repeatedly stressed, however, non-plasmolysed *Onoclea* gametophytes fail to form antheridia at any stage of development under the prevailing conditions of culture. The validity of this argument is further strengthened by the observation that plasmolysis of 5-day-old and 4-day-old prothalli also led to the first appearance of antheridium initials within three days. So far as I am aware, this is the earliest reported onset of antheridium formation among homosporous ferns without an extraneous supply of antheridial factor.

Although these results are consistent with the proposed hypothesis, the alternative possibility should also be considered that plasmolysis hastens the onset of antheridial factor synthesis. An attempt was therefore made to assay for the presence of antheridium-inducing activity. Unfortunately, it was not feasible to obtain a sufficient amount of extract from the tiny antheridium-forming individuals to assay for the presence of such activity in the prothalli. It was observed, however, that young (non-plasmolysed) *Onoclea* prothalli failed to form antheridia if they were inoculated among the temporarily plasmolysed, antheridium-forming prothalli of this same species. In contrast, young (non-plasmolysed) *Onoclea* prothalli did form antheridia if they were inoculated among antheridium-bearing prothalli of the six polypodiaceous fern species tested that formed antheridia spontaneously under the prevailing conditions of culture. All these species secrete the active substance into the medium. In the most closely investigated species, *P. aquilinum*, it was demonstrated that the individual prothallus becomes insensitive to the antheridial factor before it begins to elaborate it at an effective concentration[6]. Accordingly, the antheridia arise in response to antheridial factor in the medium (secreted there by more rapidly growing individuals of the gametophyte population[6]).

These results appear to favour the hypothesis that the temporarily plasmolysed prothalli form antheridia without the intervention of the antheridial factor, due to the decay of the postulated block to antheridium formation. Accordingly, they support the proposed hypothesis that the antheridial factor acts by overcoming a block to antheridium formation that prevents the realization of the genetic potentiality for antheridium formation. Attempts are, nevertheless, being made to design experiments that will permit an unequivocal decision between the two alternative interpretations of the results.

It is hoped that these investigations will contribute toward the solution of the problem as to how genetic potentialities for developmental change are realized at characteristic stages of development and how their realization is, at earlier stages, suppressed.

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SITES YIELDING HOMINID REMAINS IN BED I OLDUVAI GORGE

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TOGETHER with Prof. C. Arambourg and Prof. Raymond Dart, I was recently invited by Dr. L. S. B. Leakey to examine the excavations that he and Mrs. Leakey are directing in Bed I at the Olduvai Gorge, Tanganyika.

Bed I consists mainly of volcanic tuffs in the eastern part of the Gorge, but an increasing amount of clay and corresponding reduction in tuffs is observed as one goes westward, so that the westernmost exposures of this Bed consist almost entirely of clays. The tuffs are now all considered to have been laid down under water whereas the bentonitic clay layers with which they are interrelated represent still-water lake deposits. While it was thought by Reck that the coarser beds to the east accumulated fairly rapidly, the clays are considered to have formed only very slowly and in deep water. The edge of the lake shelved gradually, so that periodic fluctuation in water-level would have exposed an appreciable area of the lake margins for temporary settlement. Dr. Leakey states that the fauna includes forms related to the okapi and sitatunga, which indicate a climate wetter than that found at Olduvai to-day and sufficient to maintain swamp and rain-forest. The latter may have been relict patches of an earlier extension, since forms such as lion, cheetah, hyena, horse, hartebeeste and gazelle, which are also represented in the Bed I fauna, usually prefer savannah or grassland country.

We spent up to two days examining the horizons in which the excavations are being conducted, and I had one day in the Coryndon Museum, Nairobi, working on representative collections from the excavations. Besides the main site *FLK*.I from which the skull of *Zinjanthropus boisei* came, we examined three other excavations in Bed I (*FLK.NN*; *FLK.N*I and *MK*.) as well as four in Bed II (*FLK.N*II; *FLK*.II; *BK*.II and *HWK*.II).

At *FLK*.I the *Zinjanthropus* horizon lay on and in the top of 1–2 in. of a thin, bentonitic clay layer, and some 70 ft. × 30 ft. of this had been exposed at the time of our visit. Although most of the floor had been lifted prior to our arrival, a section some 2 ft. × 15 ft. remained, and from this and the horizontal grid plot, the nature of the floor and the position of every find could be clearly seen. The nature of the scatter pattern on these excavated floors is always the same, and the faunal and cultural material lies on the surface of the clay or in the top-most few inches. Similar bone concentrations are absent from the immediately over- and under-lying beds (yellowish tuffs).

The floors, or temporary land surfaces, are usually flat or very gently undulating, and represent exposed and dried mud-flats which became available for temporary settlement by the recession of the lake waters.

Although the usual geological signs of seasonal fluctuation of water-level are absent from the sites, yet it is hard to see how the bone on the living floors could have remained in the unweathered state in which it is found unless the surface had been fairly quickly covered again by the lake waters or unless the mineralization process had begun almost immediately. Bone lying to-day on the surface in the tropics splits up and disintegrates rapidly, and often exposure for only one or two seasons is necessary for chemical and biological agents to destroy it. I have observed that in tropical climates with alternating wet and dry seasons and high diurnal temperatures, the higher the rainfall the quicker bone is destroyed. If, however, the climate when these land surfaces were exposed had been more temperate, with more evenly distributed rainfall and lower temperatures, it is probable that the speed with which bone would be destroyed would be considerably slowed down. I observed on these floors only a few bone fragments which showed evidence of natural weathering, so that unless some such climatic adjustment is postulated it is difficult to understand how the living sites on the surface of the clays could have been exposed for any long period of time before they were again covered. Such exposure and burial must be related to temporary periodic fluctuations in the water-level, perhaps even to seasonal fluctuation, as can be seen occurring to-day around the edge of Lake Manyara, for example.

The faunal and cultural remains occur in a similar manner on each of the three areas where the old land surfaces have been excavated; that is to say, they are scattered in varying concentration over the surface. They consist of broken and sometimes complete bones as well as natural and artificially fractured stone, both quartz and lava. On *FLK*.I, the horizontal plot shows clearly a roughly circular concentration of smaller bone fragments, many small flakes and flake fragments and other worked pieces of quartz and lava over a 15-sq. ft. area. Outside this, the bone and stone occur much more sparsely, except for a rough arc of larger bone fragments and stones a few feet distant on the side from the main concentration.

At *FLK.NN*, artificially fractured and all other stone is very rare, and on some of the faunal remains the marks of teeth, radiating fractures and possible gnawing of the articular ends of long bones suggest that carnivores may have been responsible for part of this accumulation. There is no obvious evidence of carnivore (lion, leopard or hyena) activity at *FLK*.I or *FLK.N*I, however. On these sites the bone fragments consist usually of small sections, splinters and articular ends of long bones and broken mandibles, mainly of pig, antelope and horse, with some reptile and bird bone. Complete animals are never found, so that such parts as are preserved may have been obtained mainly by scavenging.

The number of animals represented on the floors was never large, but it was sufficient to suggest that the remains were carried in rather than that the floor represents a kill site. The shafts of many of the long bones have clearly been fractured to extract the marrow, and the articular ends lie discarded nearby. Sometimes shaft fragments show comminuted fracture on the interior surface, such as experiment shows results from smashing and crushing with a rounded, blunt tool.

Both the natural and artificially fractured lumps of lava and quartz, rarely more than the size of the fist, must have been intentionally carried to the positions where they were found. Every indication is that natural wash must be excluded, and there is never any sand, grit or fine gravel associated with the material on the floors—only the stones and bones lying on the clay. Moreover, as the fractures of nearly all the bones, as of the worked stone, are quite fresh, these floors must, as already stated, have been covered rapidly after their occupation.

The natural stones are likely to have been tools or potential tools just as much as the fractured ones. These last consist of gradations of 'bashing stones', from natural lumps with one or two flakes removed to full polyhedral forms; there are many flakes struck unintentionally from these. Other specimens have been intentionally flaked, usually bilaterally, to form chopping tools, and the flakes stuck from these are also likely to have been used, though, unlike those in Bed II, they show no evidence of intentional retouch or unquestionable utilization. The number and standardization of specimens clearly indicate a regular pattern of intentional manufacture. A statistical comparison of the choppers and polyhedral stones and cores with the flakes and flake fragments from these floors will be of considerable interest and may show whether any of these tools might have been made elsewhere and afterwards carried to the site.

The two horizons in Bed I (*FLK*.I and *FLK.N*I) showed every indication that they were temporary living places of the hominid which made the Oldowan implements and broke up the bones lying on these floors. The evidence we saw excludes any other interpretation. Hominid remains have now been found in intimate association at three horizons in Bed I (*FLK*.I; *FLK.NN*I and *MK*.) and, should no other more advanced form of man be found in this bed, there would be strong reason to accept *Zinjanthropus* as the toolmaker.

I wish to thank Dr. Leakey for making this visit possible and for providing opportunities for discussion with two eminent colleagues. There can be no doubt that these excavations, which have already uncovered so much—from the remains of a complete *Deinotherium*, most of the bones of which had been reduced to a pink powder, to the poison fang of a puff adder—have every possibility of finding the remains of any other hominid, should such have existed contemporaneously with *Zinjanthropus*.

LETTERS TO THE EDITORS

GEOPHYSICS

Southern Hemisphere Fall-out and the Meridional Circulation

THE interpretation of the role of the stratosphere in determining the global fall-out pattern of radioactive debris has recently moved towards a compromise of a number of models.

Libby[1] suggests that a plausible explanation of measurements of the stratospheric burden and of surface fall-out can be advanced in terms of : (*a*) Martell's theory[2] of an 8–12 month residence time for polar latitudes and one of several years for equatorial debris ; (*b*) the Machta model[3] in which the concentration of fall-out in middle latitudes is associated with a meridional circulation in the lower stratosphere[4].

Stewart[5] first suggested that the spring increase in fall-out was a result of such a mean circulation. Libby notes that this concept of stratospheric air rising at the equator, and falling elsewhere, particularly in polar regions, is consistent with an early fall-out for polar injections.

In a previous report describing measurements of total β-activity in rainwater at Melbourne[6], quantitative evidence was advanced that a mean poleward circulation in the lower stratosphere of about 1 m./sec. was largely responsible for the latitudinal spread of debris in the southern hemisphere. The existence of such a circulation is favoured on purely meteorological grounds[7,8].

Further measurements of fall-out presented here give additional support to the idea that the meridional circulation plays a significant part in determining the global fall-out pattern. The results are given in Fig. 1 in the customary units of μμc./l. (The sampling area used was $2 \cdot 2 \times 10^3$ cm.^{2}.) These are also presented in arbitrary units of quantity of material by correcting for decay following Hunter and Bellou[9], on the assumption that half the material is due to the 1958 'Hardtack' tests, and that half originated in 1955. (The latter date represents a compromise between the 1954 'Castle' and 1956 'Red-Wing' tests.) The most drastic revision of this assumption, which is based on Libby's injection results and a two-year stratospheric half-residence time, would affect the shape of the corrected curve by a negligible amount.

The most striking feature of Fig. 1 is the pronounced seasonal variation, with peaks in late 1959 and 1960 occurring roughly during the spring in the southern hemisphere. The peak in early 1959 can be associated with the initial arrival of debris from the 1958 'Hardtack' series. This seasonal variation must be regarded as a meteorological effect since no high-yield tests have been conducted in the southern hemisphere at any time. Stewart's view of an enhanced meridional circulation during the winter is therefore a most attractive explanation.

The total β-activity of fall-out during 1959 measured at Melbourne in the present investigation was 13 mc./km.^{2}, and with the above assumption regarding the time of origin of the debris, a strontium-90 fall-out of 1 mc./km.^{2} can be estimated. This agrees satisfactorily with the value of $\sim 1 \cdot 3$ mc./km.^{2} reported by the H.A.S.L. project for these latitudes[10]. During the same period, stratospheric soundings[11] indicated that the average strontium-90 concentration at 38° S. and 200 mb. was approximately 5 d.p.m./1,000 S.C.F. (that is, 2×10^{-14} c./m.^{3}). If mean vertical motion is solely responsible for transfer across the tropopause, an average vertical velocity of 200 m./day at 200 mb. can be inferred. Somewhat smaller values would be obtained at greater heights.

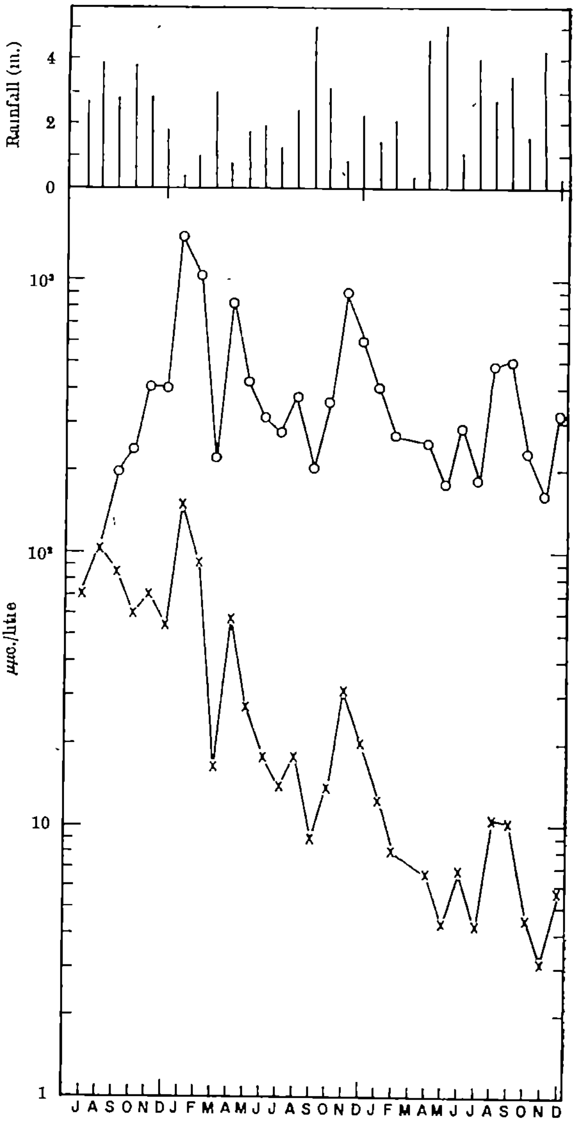

Fig. 1. Monthly fall-out data at Melbourne, Australia. ×, total β-activity ; O, corrected for decay

This is an obvious over-simplification, and estimates of mean vertical velocities of 50 m./day and 120 m./day by Brewer[4] and Priestley[7], respectively, suggest that roughly half the downward movement of debris can be attributed to mean motion and half to eddy transfer.

At our present state of knowledge of stratospheric meteorology there is considerable room for conjecture; but it seems clear that, in interpreting global fall-out, the role of the mean meridional circulation cannot be ignored.

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RADIOPHYSICS

Ionospheric Scattering of Satellite Transmissions

RADIO waves from satellites may fade in both regular and random fashion when received with a linearly polarized aerial. Fading due to Faraday rotation or satellite rotation is an example of the former type. Random fades, up to several per second, are sometimes observed, and these have been attributed to scattering from the same kind of ionospheric irregularities as produce radio star scintillation. This fading is more rapid for satellites than for radio stars since it depends on satellite velocity rather than ionospheric drift velocity. Henceforth we shall be concerned only with the randomly fading component.

The 40 Mc./s. transmission from *Sputnik I* has been recorded at Cambridge by Kent[1]. It was observed to fade rapidly at night, but only when the satellite was to the north of Cambridge. The transition to or from this rapidly fading condition was quite abrupt. Kent has concluded that the irregularities which cause the fading are present only to the north of Cambridge; but Mawdsley[2] has suggested that the effect is a natural outcome of the anisotropic nature of irregularities which appear to be aligned with the Earth's magnetic field. Thus, generally speaking, strong forward scattering should be observed only when the satellite is poleward of the observer.

We have recorded the 19·990 Mc./s. transmission of *Explorer VII* at Wellington, New Zealand. Observations commenced recently when details of this satellite came to our notice through the *International Geophysical Year Bulletin*, No. 29, of the U.S. National Academy of Sciences. Table 1 lists the passes during which recordings were made before the orbit moved completely into daylight conditions over Wellington. Scintillations were observed only when the satellite was to the south at night. The transition was abrupt.

Table 1

Date	Local time of pass (approx.)	Remarks
3 6.60	2052–2100	Scintillations when satellite to south
,,	2235–2245	,, ,, ,, ,,
4.6.60	2027–2036	,, ,, ,, ,,
7 6 60	1357–1404	No scintillations
,,	1541–1550	,, ,,
,,	1728–1735	,, ,,
,,	1913–1925	,, ,,
,,	2100–2110	Scintillations when satellite to south

A search of the literature has since brought to light several instances of similar behaviour. They are as follows:

(1) Kraus and Albus[3] made signal strength and Doppler frequency shift measurements of the 20·005 Mc./s. transmission from *Sputnik I* at Columbus, Ohio. Scintillations were recorded as the satellite rose in the north, ceased abruptly as the satellite approached the station, and were absent for the rest of the pass. This behaviour was not apparent on south-to-north passes. It was suggested that an ion cone might precede the satellite, forming an unstable medium for waves passing through it. More recently, Hame and Kennaugh[4] have extended these observations to the 20·005 Mc./s. transmission from *Sputnik III*. They find that scintillation occurs only when the satellite is north of Columbus irrespective of the direction of pass. They have suggested an explanation in terms of changes in magneto-ionic propagation as the Earth's magnetic field varies according to the position of the satellite. It is difficult, however, to see how to explain a change from regular to random fading in this way.

(2) Recordings of signal strength, Doppler frequency shift, and bearing angle of the 20·005 Mc./s. transmission from *Sputnik I* have been made at Komuro, Japan, by Miya *et al.*[5]. Referring to Fig. 2 of their paper, a sudden change in fading pattern was recorded when the satellite was near the station. South of this point the random fading is small compared with Faraday fading. The more intense rapid fluctuation when the satellite is in the north could perhaps be due to scintillation if the recording system had the characteristic of a low-pass filter. Miya *et al.* ascribe the transition as one between line of sight propagation and regular ionospheric propagation associated with ground scattering. However, the transition of Fig. 2 of their paper occurs when the satellite is quite close to the station. Fig. 6 shows fluctuations in bearing angle when the satellite is 'in view' north of the station. The fluctuations disappear as the satellite moves to the south.

(3) Fluctuations in Doppler frequency change at Portsdown, England, of the 20·005 Mc./s. transmission from *Sputnik III* have been noted by Kitchen and Joy[6]. Fig. 3 of their communication shows an example of this behaviour, which is apparent only to one side of the station. The fluctuations have been attributed by these workers to small-scale *F* region irregularities.

(4) Sudden fluctuations in the signal strength at Kiruna, Sweden, of the 20·005 Mc./s. transmission from *Sputnik III* have been reported by Liszka[7]. Fig. 1 of his communication suggests that the fluctuations may be asymmetric with respect to the observing station. Because these fluctuations occur at similar positions for successive orbital revolutions, Liszka has suggested that long-lived heavily ionized tracks are produced by the passage of the satellite through the

atmosphere. This repetitive spatial occurrence is, however, a natural feature of scattering sensitive to aspect from irregularities aligned with the Earth's magnetic field. Although *Sputnik III* may not have passed north of Kiruna (geographically) it is to be noted that, because the magnetic field lines there are nearly vertical, energy may still be scattered from a satellite in the south to an observer in the north.

(5) More recently, Yeh and Swenson[8] have undertaken a detailed analysis of scintillations, observed chiefly on the 20·005 Mc./s. transmission of *Sputnik III*, at Urbana, Illinois. Night scintillation is associated with *F* region and is observed only when the satellite is in the north.

In view of the possible association with radiation belts[9] it would be useful to have scintillation observations over an extended period covering a wide range of latitudes. Few radio stars are suited to this purpose, particularly for the southern hemisphere. Thus, although it may be desirable from some points of view to interrogate the majority of future satellites, there is a strong case for providing a few which transmit continuously at a frequency between about 20 and 40 Mc./s. for some time to come.

In conclusion it appears likely that the night-time scintillation of satellite transmissions demonstrates the ability of field-aligned irregularities to scatter metre wave-length radio waves in a manner highly sensitive to aspect. This is not to be expected of such gross features as are deduced from radio star scintillation or from Kent's observations. Some fine structure must be sought, such as might arise when aggregates of charged particles spiral into the atmosphere along magnetic field lines and create ionized tracks.

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ASTROPHYSICS

Galactic Motions in a Large-Scale Magnetic Field

Recent observations on spiral galaxies have shown that their interstellar material cannot be in purely rotational motion. The evidence points to a circulation of interstellar gas between the galactic plane and the galactic halo.

It has been shown for several galaxies[1], including our own[2], that the observed differential rotations will, within less than 10^8 years, transform an initially structureless interstellar cloud into a spiral arm having a pitch like that of observed arms. Since spiral structure presumably persists for longer than 10^9 years, it was suggested that arms may somehow be preserved to 'look young'[2]. However, I[3] have shown that one likely mechanism, magnetic fields embedded in the arms, cannot achieve this; on the contrary, these fields disperse an arm within 10^9 years by their lateral magnetic pressure and an associated transfer of angular momentum. Other evidence that the arms are actually young comes from the observations of Rougoor and Oort[4] that the '3-k. parsec arm', an arm of neutral hydrogen about 3 k. parsec distant from the galactic centre, moves radially outwards with a speed of roughly 50 km./sec. Similar motions have been observed by Munch[5] in the galaxy *M*31. According to observations by Pismis[6] and by Kerr[7], even the well-established spiral arms in our galaxy are moving away from the centre with a velocity of roughly 5 km./sec. At this rate no arms of interstellar gas can remain in the galactic plane after 2×10^9 years unless new arms are formed near the centre.

If the 3-k. parsec arm is a continuing or recurrent phenomenon, the galactic centre is an insufficient source of gas[4]. Furthermore, the 3-k. parsec arm demands a source of angular momentum since it participates in the local circular motions and the angular momentum involved in these motions increases outwards[4]. Hoyle and Ireland[8] investigated the effect of interstellar magnetic fields, but they assumed that the angular momentum gained by the 3-k. parsec arm is derived from elsewhere in the galactic plane. This implies that another cloud of gas should approach the centre, which is not observed. We must, therefore, look to the galactic halo as a source of both gas and angular momentum. The drain of gas from the halo near the centre and the radial motion in the plane imply a return of the gas to the halo. As suggested by Hoyle and Ireland[9], this may occur by means of magnetic bubbles rising from inter-arm regions.

Therefore, both observational and theoretical arguments indicate the existence of a large-scale circulation of the interstellar gas and of its angular momentum between the galactic plane and the halo. Such a circulation can be driven only by interstellar magnetic fields. Davis and Biermann[10] have recently discussed possible models of the galactic halo. They suggest that field strengths of 2×10^{-5} gauss in the plane and 6×10^{-6} gauss in the halo and a gas density of 10^{-2} hydrogen atom per cm.^{3} in the halo are compatible with observations and theory. Although a detailed model of the circulation would be very premature (even the very similar problem of the hydromagnetic dynamo has not been solved satisfactorily), some features may be pointed out which may guide further tests on the possibility of the proposed circulation.

First, superposed on any local turbulence there must be magnetic and velocity fields of a much larger-scale size, of the order of several kiloparsecs. Because of the large electrical conductivity the energy involved in these fields will be dissipated only through interaction with the small-scale turbulence. This is probably small, so that the circulation can be maintained over times longer than 10^9 years and thus preserve the spiral structure of the galactic plane.

Second, since the gas density decreases away from the plane much more rapidly than the field strength, the large-scale field in the halo can control the motion of the gas. This may permit the 'unwinding' of the magnetic fields which have been wound up by differential rotation in the plane and have risen into the halo together with the gas in which they are embedded. The gas being dispersed throughout the halo must stream predominantly along lines of force since the field strength would otherwise decrease proportionally to the gas density. Since the supply of gas and of angular momentum in the halo is comparable to that in the plane, the unwinding of the field and the dispersal of the gas need not occur very rapidly. Probably the field will adjust itself to a rather force-free geometry. But as Schluter[11] has shown, even a perfectly force-free magnetic field can transport momentum and angular momentum. Hence a circulation of energy and of angular momentum appears possible, with the gas moving radially outwards in the plane, gaining energy and angular momentum from gas moving inwards through the halo.

Finally, I have attempted to make some estimates on the forces acting on the gas at the centre and in the 3-k. parsec arm. For this purpose I assume that the large-scale magnetic field within regions of high gas density is axisymmetric, although no such symmetry is to be expected outside these regions. The assumption is justified primarily by the conclusions which can be drawn from it. Let R and θ denote the distance from the centre and the angle in the direction of the galactic rotation. The rate of transfer of angular momentum to a unit mass of gas in the galactic plane at a density ρ is (in c.g.s. and gauss units):

$$\frac{d}{dt}(Rv_\theta) = \frac{H_R}{4\pi\rho}\frac{d}{dR}(RH_\theta)$$

On the assumption that the field lies parallel to the trailing arms we find that H_θ and H_R have opposite signs. The quantity $|RH_\theta|$ must attain a maximum at some $R = R^*$ since it vanishes at the centre and decreases outwards at the edge of the galactic plane[12]. The gas within R^* loses angular momentum and must be moving inwards; the gas outside R^* gains angular momentum and moves outwards. Hence gas must condense on to the galactic plane near R^*. Presumably it moves at different rates on either side of R^*. I conclude that on each side of the galactic plane there must exist two circulations which meet on a surface through R^* but do not interfere with each other. This implies that the magnetic fields are self-contained and angular momentum is conserved in each circulation. The neutral hydrogen within 1 k. parsec from the centre is observed not to expand with the surrounding 3-k. parsec arm[4]. Accordingly, I identify $R^* = 1$ k. parsec and make the following numerical estimates for the outer ($R > 1$ k. parsec) circulation, which involves all the spiral arms: near the Sun, $H_\theta = 2 \times 10^{-5}$ gauss; near $R = 4$ k. parsec, $H_\theta = 4 \times 10^{-5}$ gauss (negligible transfer of angular momentum outside $R = 4$ k. parsec); and at the 3-k. parsec arm, $H_\theta = 7 \times 10^{-5}$ gauss, $H_R = -0{\cdot}25\ H_\theta$, $\rho = 0{\cdot}4$ hydrogen atom per cm.[3]. The resulting transfer of angular momentum permits the 3-k. parsec arm to move radially outwards with a speed of about 50 km./sec. and still to participate in the local circular motions. Thus the assumption of axisymmetry within regions of large gas density results in the explanation of the isolated central region and of the velocity of the 3-k. parsec arm. Furthermore, these estimates imply that gas may rotate more slowly than stars at the same R, since the gradient of the magnetic field causes forces on the gas which partly balance the galactic gravitational attraction.

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Some Solar Lines in the Infra-Red Spectrum observed from a High-Flying Aircraft

In a previous communication[1], it was pointed out that strong absorption by atmospheric constituents obscures large parts of the infra-red spectrum from ground-level observation. However, by working at high altitudes and by using a high-resolution spectrometer, it is possible to observe additional features due to absorption in the solar atmosphere. Among these are two absorption lines due to the electronic transitions in solar hydrogen 3–4 and 4–8, which occur in the great water-vapour band at 1·9μ.

These lines, which have not previously been detected because of obscuration by water-vapour absorption—even at mountain observatories—are clearly observed at 40,000 ft., as shown in Fig. 1. They remain of the same intensity at higher altitudes, thus confirming their solar origin.

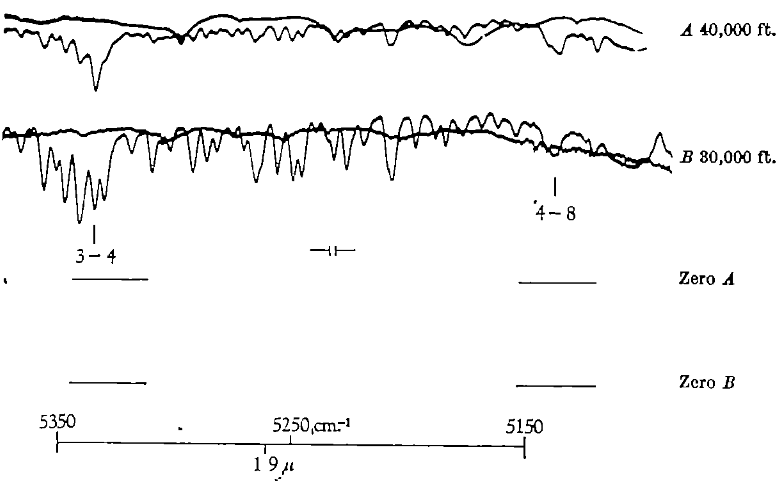

Fig. 1

Another solar line which we have now detected at high altitudes is the 4–6 transition, lying in the $2\cdot6\mu$ water-band. The presence of this line will produce slight corrections to some of the water-vapour concentrations calculated by Houghton and Seeley[2].

Full results of our measurements are being submitted to the Royal Society for publication in the *Philosophical Transactions*.

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PHYSICS

Line-Spread Functions of Photographic Emulsions

The detail-recording ability of a photographic emulsion may be tested by exposing it through a very narrow slit, and, after processing, measuring the variation of density with distance, x, from the centre line of the image. When the scale of density is transformed into a scale of effective intensity of illumination, the result is the line-spread function, $B(x)$.

Traditionally, the 'double-exponential' function[1-3] is taken as a convenient model for the analytical representation of measured results. For the present purpose, this function may be expressed as :

$$B(x) = \pi F \quad \exp(-2\pi F|x|)$$

Its Fourier transform, the sine-wave response function, is :

$$Y(f) = 1/\{1 + (f/F)^2\}$$

where f is the spatial frequency and F is the value of f such that $Y(F) = 0\cdot5$. In generalized form, these functions are shown as the dashed curves in Figs. 1 and 2. Some workers, however, have indicated dissatisfaction with this model, referring either to its physically unlikely sharp peak and central discontinuity or to the related slow fall of sine-wave response at the higher frequencies. Various modifications have been proposed, but most involve at least one additional parameter.

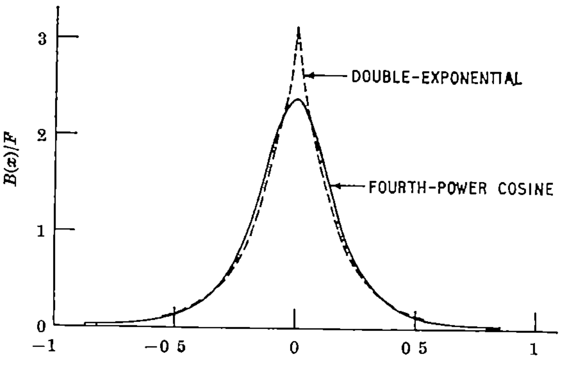

Fig. 1. Line-spread functions

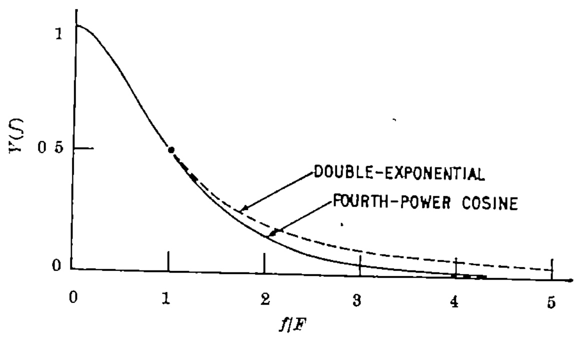

Fig. 2. Sine-wave response functions

In the course of work on television image structure, it has been noticed that the 'fourth-power cosine' function, as it may be termed, offers advantages as a model line-spread function. Expressed in terms of a length parameter, D, the function is :

$$\begin{aligned} B(x) &= 2\cos^4\{\tan^{-1}(x/D)\}/\pi D \\ &= 2/\pi D\{1 + (x/D)^2\}^2 \end{aligned}$$

or, in terms of the parameter F already defined :

$$B(x) = 2AF/\pi\{1 + (AFx)^2\}^2$$

where $A = 3\cdot7437$. The corresponding sine-wave response function is :

$$\begin{aligned} Y(f) &= (1 + 2\pi Df)\exp(-2\pi Df) \\ &= (1 + 2\pi f/AF)\exp(-2\pi f/AF) \end{aligned}$$

Generalized in terms of F, these functions are shown as the full-line curves of Figs. 1 and 2.

Comparing the curves in Fig. 1, it will be seen that the fourth-power cosine function is closely similar to the other in the region of the 'skirts', but it has the desirable property of being less-sharply peaked and there is no central discontinuity. The related features in Fig. 2 are the similarity at the lower frequencies and the more-rapid fall at the higher frequencies.

For describing line-spread functions in general, the half-intensity width, W, is often a useful parameter. In symmetrical functions, W is defined by $B(W/2) = 0\cdot5$ whence, for the fourth-power cosine function :

$$W = 0\cdot3438/F = 1\cdot2872D$$

Taking a medium-speed panchromatic film as an example, it is believed that W might typically be 15μ, whence $F = 23$ lines/mm. and $D = 12\mu$. A fair approximation to the subjective resolving power determined with a high-contrast target is often given by $1/W$; this is 67 lines/mm. in the example.

If the proposed function proves in practice to be a good model, its relation with the well-known $\cos^4\theta$ law of off-axis illumination in an optical imaging system will be of interest. However, whether or not the θ of that law can be identified with the $\tan^{-1}(x/D)$ of the expression for $B(x)$ first given, it may possibly be significant that the distance D appears to be about equal to the emulsion thickness.

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Detection of Free Charges from Motor-Car Exhaust Gases

The production of charged entities during combustion is well known[1], and some of these entities have proved to be gaseous ions of low molecular weight containing carbon and hydrogen or oxygen and hydrogen[2]. It is known that ionization takes place in explosion motors and it is used, for example, to detect ignition induced by deposits[3]. Jet aeroplanes are reported to become charged electrically[4]. Part of the charging current (about 500 μamp.) might be due to the preponderance of free charges of a certain sign in the exhaust gases.

In an investigation of the role of unipolar radical ions in radiation chemistry and their radiomimetic properties and possible role in the initiation of lung cancer[5], I have done some preliminary work on the presence of free charges in motor-car exhaust gases and from other sources. The apparatus consisted of a charge collection chamber with a geometry rather similar to that used by Siksna[6] (see Fig. 1). The unit is easily portable (the high-tension device is made with transistors). Careful tests have been done to ensure that creep currents are negligible even under very moist conditions. To this end, the 'Teflon' insulator is heated electrically. The output of the instrument is proportional to the concentration of charge in the gas. In more stationary cases, I have used a large parallel-plate condenser[7] (plate separation distance, 100 mm.; size 300 mm. × 300 mm.) to measure the total current that can be collected, by applying a field-strength of 300 V./cm. This unit measures total rate of production of charge rather than concentration of charge.

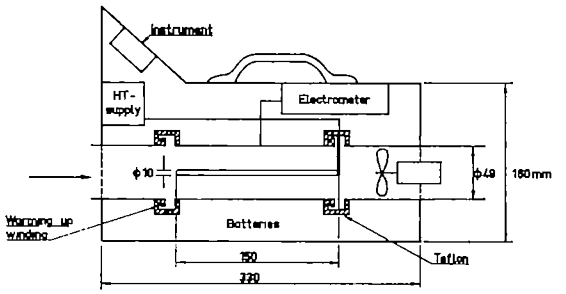

Fig. 1. Portable charge collection apparatus. Air velocity 1 m /sec. Transistorized high-tension variable, 700–1,200 plus or minus. Charge collected at outer electrode is amplified by a conventional electrometer tube circuit. The insulator is heated by mercury batteries. All dimensions are in millimetres

Virtually every car tested (about forty so far) exhibited charge production. On starting the engine, the production is low but increases to high steady states (often ~ 10^7 charges/cm.^{3} at full throttle. The total current collected varies from car to car. In the worst case, a current of 100 μamp. was detected, or more than 10^{14} charges/sec. The positive and the negative parts were nearly equal. In this vehicle, the silencer was in bad condition. Small motors, for example, from 'mopeds', were found to yield rather low currents, namely, about 10^{-9} amp., or slightly more than a cigarette[5]. The charges are confined to the exhaust gas cloud. The concentration drops steadily with distance from the exhaust tube. Nevertheless, I have detected charge concentrations on the pavements of streets with heavy traffic and in the back-seats of cars driving behind Diesel trucks. Starting a car in a garage fills the space rapidly, with a charge concentration which, however, drops in some tens of seconds if the car leaves the garage. The charge monitor is provided with variable collector voltage so that some information on the mobility spectra can be obtained. However, the conditions have not been steady enough to facilitate observations of this kind.

The level of the concentration is roughly the maximum that could be expected from a gas-ion recombination[8], but we are not justified at present in making any exact statements on the nature of the charges detected. There are strong arguments[2] for believing that molecular gaseous ions are produced in explosion motors. The fate of these ions will, however, be dependent on the properties of the exhaust tube, the presence of filters (it is known that wool eliminates most of the ions[9]), the humidity and presence of water or oil droplets, dust, etc. The latter may sorb a large part of the original ions. Further work will have to be done to explore the nature of the charges at different times. It is likely that free radicals can be detected by condensing rapidly the exhaust gases from explosion motors, as is the case for cigarettes[10]. In many other instances, apart from explosion motor exhaust gases, charges have been detected, for example, in gas and electrical welding, smoking of cigarettes and cigars[5], household cooking gas and flames and glowing bodies (such as bread-toasters).

Further study of the nature of these charges would seem to link radiation chemistry and radiation biology with the field of air pollution and public health in an interesting way. If it turns out that the charges represent radiomimetic entities, much can be done to reduce them to low levels, for example, by filtering electrically the inlet and outlet gases in motors.

I am indebted to the Swedish Cancer Society for economic support. Advice by Dr. R. Siksna on the charge collection chamber is also gratefully acknowledged

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GEOCHEMISTRY

Apparent Geochemical Association of Bismuth and Thallium

The geochemical association of certain pairs or groups of elements has been noted by several authors, and a classical example is the rubidium/thallium relationship which has been described by Ahrens[1]. In order that such a coherence should be established, it is necessary that not only should a large number of accurate analyses be available but also that these data should be obtained for each element on identical geological specimens.

Until recently, very few data have been available for the abundance of bismuth in silicates due to the lack of analytical methods sufficiently sensitive for its determination at its low concentration levels in such rocks. However, a new method has been developed[1-4] in which this element has been determined spectrochemically after previous enrichment by an anion-exchange procedure. It has also been possible to determine simultaneously thallium and other elements, and abundance data for these two elements have been obtained for some sixty rock specimens. In addition to this work, Ehmann and Huizinga[5] have determined bismuth and thallium at very low concentrations in chondrites by a neutron activation method. The only other simultaneous analytical data for bismuth and thallium in silicates are those of Preuss[6], who estimated these elements by a spectrochemical distillation technique.

I have collated all the available data and noted a surprising apparent relationship between bismuth and thallium, and this is shown in Fig. 1, where a log/log plot of thallium concentrations as a function of bismuth concentration is shown. The points on the diagram for Goldschmidt[7] and the Noddacks[8] are estimates only, and one of my points represents the average of a suite of 19 dolerites from the Jagersfontein diamond mine, South Africa, of which some of the individual analyses show greater scatter than the other points on the graph. All the data include a comprehensive range of silicate rocks from granites to ultra-basics.

The apparent coherence between bismuth and thallium is difficult to explain but it is interesting to note that Oftedal[9] has determined both bismuth and thallium in several samples of galena where both elements are found to be highly concentrated and has reported maxima of 500 p.p.m. thallium and 1,000 p.p.m. bismuth. Goldschmidt[7] has suggested that the enrichment of these metals in lead minerals may be due to the replacement of two atoms of lead by one atom each of bismuth and thallium. The ionic radii of lead, bismuth and thallium are shown in Table 1.

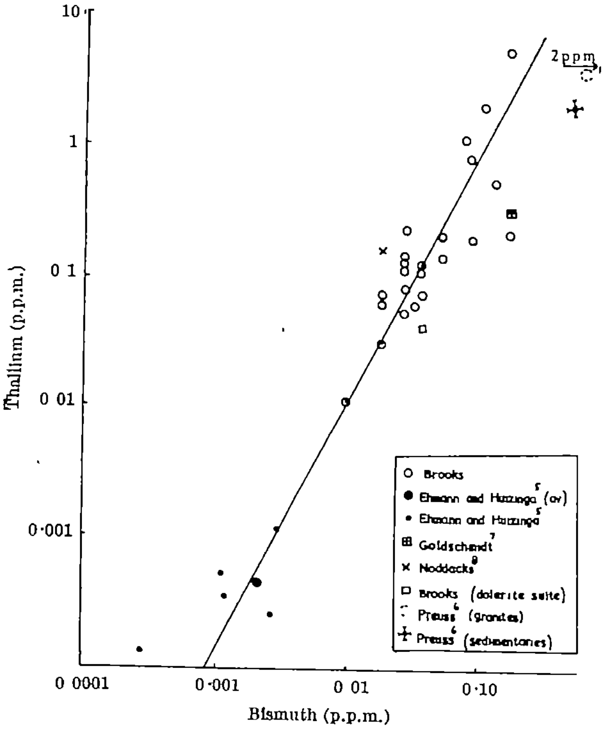

Fig. 1

Table 1. IONIC RADII OF BISMUTH, THALLIUM AND LEAD

Element	Ionic radius
Tl^+	1·49 Å.
Bi^{3+}	1 09 Å.
Pb^{2+}	1 32 Å.

The belief that lead may be the common link between thallium and bismuth is strengthened by consideration of the abundance of lead in silicates[10] where the lead concentration increases regularly from about 3 p.p.m. in ultra-basics to about 20 p.p.m. in granites. It is true that lead is not a major constituent of most rocks ; but since its abundance is very much greater than that of either bismuth or thallium, it is possible that the mechanism of a major element camouflaging a trace element will apply in this case. It is noteworthy that thallium, bismuth and lead are the heaviest three non-radioactive elements, and this may have some significance for their apparent relationship.

Therefore, it may be possible to account for the apparent thallium/bismuth coherence from the premise that one atom each of bismuth and thallium replace two atoms of lead, since the ionic radii of these three elements do not preclude this replacement.

As more data become available for the relative abundances of bismuth and thallium, it should be possible to establish further this apparent relationship.

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OCEANOGRAPHY

Overflow of Cold Deep Water across the Iceland–Greenland Ridge

INCREASING attention has been given in recent years to the overflow of cold bottom water, formed by winter cooling in the Norwegian Sea, across the various submarine ridges separating the basin of the Norwegian Sea from that of the Atlantic Ocean. In June 1960, under the auspices of the International Council for the Exploration of the Sea, nine ships from five different countries investigated the overflow across the Iceland–Faroe Ridge by means of a series of parallel hydrographic sections between Iceland

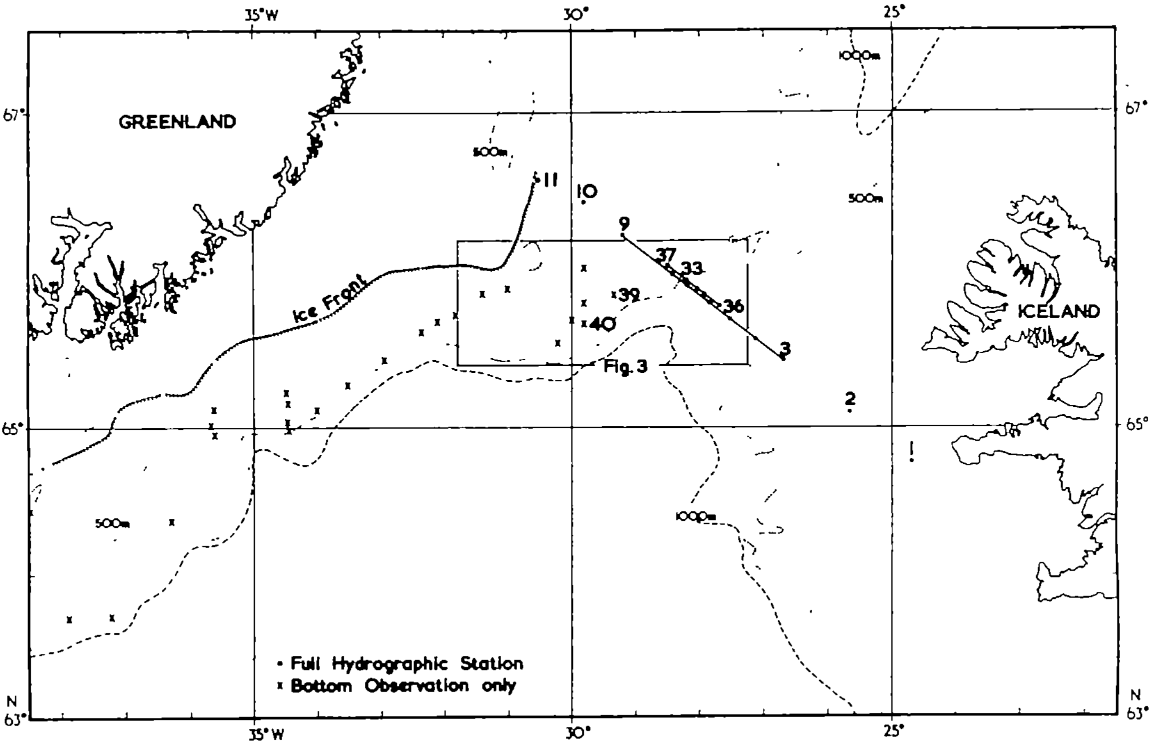

Fig. 1. R.V. *Ernest Holt* Cruise VI 1960 : Location of stations worked in the Denmark Strait

and the Faroes, and made direct current measurements at a number of strategic points.

In August 1960, the English Fishery Research Vessel, *Ernest Holt*, which had participated in the Iceland–Faroe Ridge Survey, worked a hydrographic section across the Denmark Strait (Fig. 1), along the submarine ridge joining Iceland to Greenland, and made direct measurements of the current near the bottom at a selected point along this section. The section was worked on August 15 and 16 from a position some sixteen miles from the Iceland coast to the ice front, approximately eighty miles from the Greenland coast. In the deepest part of the section, that is, below 450 metres, where any overflow taking place would be expected, the coldest bottom water found was 2·5° C. at station 7. Fig. 2*a* shows the central and deepest part of the section.

On the completion of the section, the *Ernest Holt* returned to the position, as near as could be determined, of station 7 to anchor in 550 metres depth (station 33) and make direct measurements of the current near the bottom. It was found that the bottom temperature was now 0·1° C. The time interval between the taking of the bottom temperatures

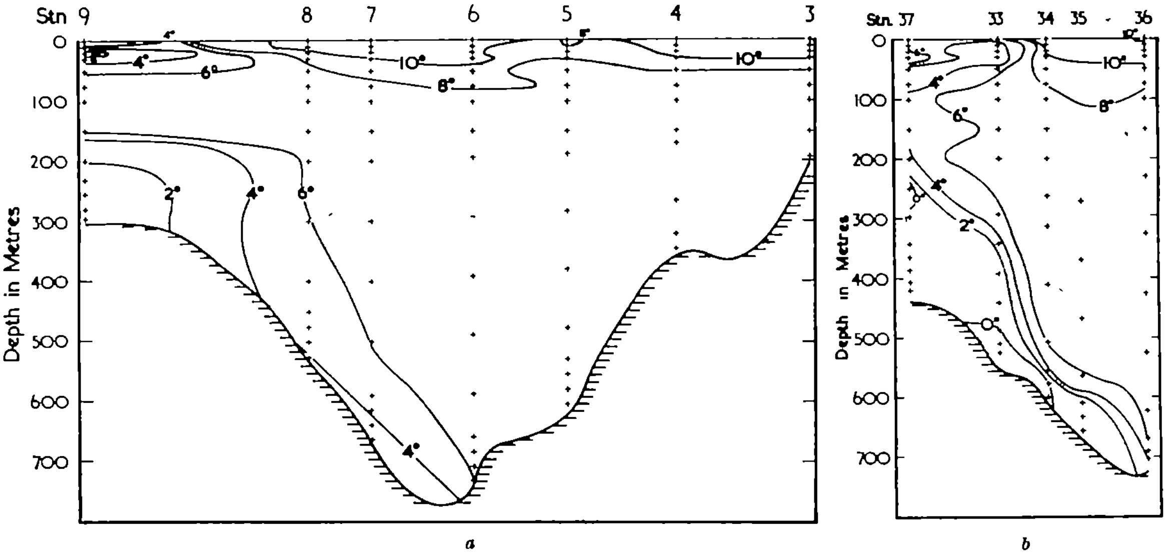

Fig 2. (*a*) Iceland-Greenland Section (1600 hr. G.M.T. August 15–1700 hr. August 16): temperature (°C.). (*b*) Part of Iceland–Greenland Section (1300 hr. G.M.T.–2200 hr. August 17). temperature (°C.)

at stations 7 and 33 was 20 hr. Direct current observations (ten in a period of 14 hr.) were then made within five metres of the bottom by means of a Carruthers' Pisa current indicator[1]. The preliminary analysis of the observations shows a residual current of 0·3 knot in a direction 250° true when the tidal current is eliminated.

Immediately the current observations were completed at 1300 hr. G.M.T. on August 17, the part of the section previously worked in the immediate vicinity of station 7 was repeated in an attempt to delimit the extent of the cold bottom water encountered at station 33 during the measurement of the current. Fig. 2*b* shows the results of this.

After this part of the section had been repeated, a bottom temperature survey on the fishing banks to the west and south of the section was commenced, during which the front of the advancing cold bottom water was identified as lying between stations 39 and 40, sixteen nautical miles apart, at 0430 hr. on August 18, as shown in Fig. 3. The rate of advance of the front of the cold water can be estimated between two limits from the knowledge of the interval of time during which it passed station 7 and the two positions between which it was situated at 0430 hr. on August 18, assuming the axis of flow to have been along the line joining stations 33, 39 and 40. The limits thus determined are 0·55 knot and 1·4 knots, and the line of the axis of flow thus assumed is approximately 255° true. This direction agrees almost exactly with that of the current measured at station 33 and, although both limits of the rate of advance of the cold water front exceed that of the observed current, the lower limit is of the same order of magnitude.

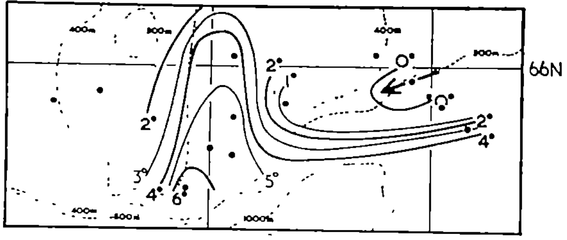

Fig 3. Bottom temperature distribution (° C.) on the south-western side of the Iceland–Greenland Ridge (1300 hr. G.M.T August 17–1600 hr. August 18)
Bottom residual current, ←●—; ●, station

An analysis of the salinity values in the deeper layers of stations 7 and 33 shows the sudden decrease in temperature to be the result of a renewal of the overflow of cold Norwegian Sea deep water across the Iceland–Greenland Ridge. This finding adds support to the hypothesis of Cooper[2] that the overflow is intermittent and takes the form of a series of discrete boluses. It is also in agreement with one of the findings of the Iceland–Faroe Ridge Survey of June 1960, namely, that the bottom temperature on the southern side of these ridges can change rapidly. It is perhaps of significance in a consideration of the factors controlling the overflow that the surface front between the warm Atlantic water and the cold southward-flowing East Greenland Current apparently shifted towards the south-east between the two occasions when the section was worked. As a result of this, on the first occasion when there was little evidence of any overflow, station 7 was located in the warm Atlantic water, but when the *Ernest Holt* returned to station 33 and found a strong overflow it was located in the cold East Greenland Current.

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LIMNOLOGY

A Second Lake with Old Sea-Water at its Bottom

FOUR years ago I directed attention to a Lake (Rørholtfjorden, part of Lake Tokke, in southern Norway) with trapped sea-water[1]. The surface altitude of that Lake is 60 m. above sea-level, and the estimated age of its bottom waters some 6,000 years, that is, of the marine salts contained in them ; the present bottom waters probably possessing one-half of the salinity of those originally isolated.

While there are a large number of land-locked waters still having some communication with the sea, there are very few where bottom waters have been completely isolated, and not washed out through admixture with fresh water layers above. Until their discovery in Lake Tokke, salt bottom waters were known only from lakes near sea-level, where isolation through post-glacial land rise must have occurred but recently.

Since my communication, work in progress on Lake Tokke has led to some results, especially concerning isotope distribution in sediments.

Further research made it very desirable to find a second lake with old sea-water, and finally, my collaborator, Mr. H. V. Søvik, in January 1961, discovered such a lake, Botnvatn, in the district of Salten, northern Norway (67° N.), the altitude of the lake surface being 12 m.

Lake Øvrevatn, a land-locked fjord[2] near Botnvatn, with an average height of its water-level of about 2 m. above daily ebb, is at the point of being isolated from the sea. We can thus assume a land rise since the isolation of Botnvatn, of about 10 m., and that the isolation probably took place some 3,000 years ago.

Botnvatn is 113 m. deep, with salt waters from 102 m. downward. Hydrography is very similar to that of Lake Tokke, where salt waters extend from 134 m. to the bottom (147 m.). A comparison may be made between the salt-water layers of the two lakes :

	Tokke, 144 m.	Botnvatn 111 m.
Chlorine (mgm/l)	9 24	7 26
Salinity (gm./kgm.)	16·71	13 14
Temp. (° C.)	5·20	4·75

As is the case in Lake Tokke, the salt bottom waters contain enormous amounts of methane, and bubble violently when brought to the surface. We thus have to face the same problem as in Lake Tokke, that with decrease in pressure the release of methane, also within the sediments, makes it very difficult to secure undisturbed samples of the bottom deposits.

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CHEMISTRY

Vibration Frequency and Ionization Potential: Halide Molecules

It has been observed that a systematic correlation is possible between the sum of normal vibration frequencies of a molecule and the first ionization potentials of its constituent atoms.

The sum, $\Sigma\nu$, of all the normal frequencies (degeneracies, if any, included) of a molecule RX_n, where X is a halogen and R a molecular group, has been found to be a linear function of the sum of the first ionization potentials, ΣI_x, of the halogen atoms occurring in the molecule. A plot of $\Sigma\nu$ for a group of molecules with the same R, but with all possible halogen substitutions against ΣI_x, is thus a straight line. Preliminary tests, indicating the validity of the relation, have been made on fifteen CX_4, nine CHX_3, six CH_2X_2, four CH_3X, nine C_2X_4, seven C_2HX_3, five $C_2H_2X_2$, three C_2H_3X, ten $^{11}BX_3$, ten $^{10}BX_3$ and three XCN type molecules.

It is also observed that the $\Sigma\nu$ values of two similar sequences of halides are linearly related:

$$\Sigma\nu(R'X_n) = \alpha\Sigma\nu(RX_n) + \beta$$

provided both the series contain the same number of halogens and the corresponding members are compared.

Details of the work and results of allied investigations will be reported elsewhere.

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Delayed Gas Evolution from Passive Films formed in the Kolbe Reaction

During the course of electrochemical kinetic studies on the Kolbe reaction, we have observed a remarkable phenomenon, the details of which are recorded here. The classical Kolbe reaction involves the anodic electrolytic synthesis of hydrocarbons from solutions of salts of appropriate aliphatic carboxylic acids. As a model system for preliminary investigations of the electrochemical kinetics of this reaction, we have examined the anodic reaction which occurs in the electrolysis of a solution of potassium formate in pure anhydrous formic acid; in this case 1 mole of carbon dioxide is evolved in the passage of two Faradays.

During steady-state electrolysis of the above solution at platinum, gold and palladium electrodes, the only gas evolved is carbon dioxide. The overall reaction is hence formally[1]:

$$2HCOO^- \rightarrow HCOOH + CO_2 + 2e$$

On interrupting the current at platinum and gold electrodes, the gas evolution ceases immediately in the normal way. However, on interrupting the current at a palladium electrode, the gas evolution initially ceases, but is then followed by a further burst of gas from the surface after a delay period of up to 5 sec., this gas evolution occurring in the complete absence of current-flow. The effect at palladium is quite reproducible and is best observed at a current density greater than 10^{-1} amp./cm.^{2} in 5-M solutions of potassium formate in formic acid (pre-saturated with carbon dioxide) at 5° C. Although carbon dioxide is evolved at platinum and gold with the same current efficiency (about 90 per cent) as at palladium, the delayed gas evolution is not observed, and the effect hence arises from some property of the palladium anode. No hydrogen is evolved as might be expected by analogy with the coupling reaction occurring with acetic and other aliphatic acids, but this is understandable since any H radicals generated by the anodic decarboxylation will ionize rapidly at a rate of at least 10^{10} amp./cm.^{2} at the high anodic potentials (about 1 2 V. with respect to the hydrogen electrode in the same acid solution) required for significant rates of electrolysis of the formic acid. Hence it is improbable that hydrogen produced in the decarboxylation step and taken up into the surface regions of the palladium is responsible for the effect, and in fact the coulombic yields of carbon dioxide gas by continuous and repeatedly interrupted polarization are identical within experimental error and no hydrogen is detectable by chemical or gas chromatographic means under either set of conditions of polarization.

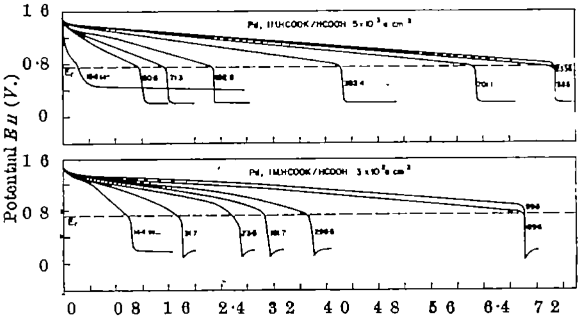

Fig. 1. Open-circuit e.m.f. decay curves taken on an oscilloscope at two current densities of prior polarization for the indicated times (sec.).

Rapid ciné-photography of the palladium surface with a simultaneous recording of the moment when current stops flowing has enabled the delay-times in gas evolution in interrupted polarization experiments to be measured with some precision to about 0·1 sec. The delay-time in the gas evolution after the current stops is found to be a function of the time of previous anodic electrolysis up to periods of about 5 min. This suggests that the anodic electrolysis

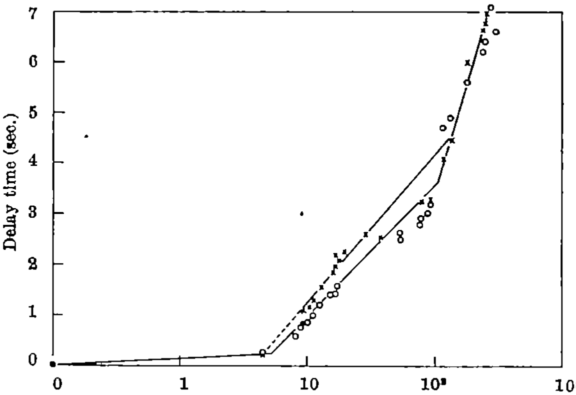

Fig. 2. Relationship of both the delay in open-circuit gas evolution (from ciné photography) and the potential delay to the period of prior anodic polarization at 10^{-1} amp./cm.$^{-2}$. ○, delayed gas evolution, ×, potential delay; 5 M HCOOK/HCOOH

forms a relatively thick film of a surface palladium formate (possibly non-stoichiometric[2,3]) at the electrode, and, after net current ceases to flow, this film breaks down auto-catalytically with evolution of carbon dioxide. The formation of this film appears to be analogous to that of passive films formed anodically, for example, in aqueous solutions at nickel and iron. Determinations of the galvanostatic charging and open-circuit decay curves, using a cathode ray oscilloscope, for the polarized palladium and platinum electrodes indicate the formation of surface films as shown in Fig. 1. The pseudo-capacity associated with the film at palladium is much greater (10–50 times) than that at platinum, and this is evidently the reason why the delayed decomposition is observable at the former metal although it may still occur but not be visible at platinum or gold. We believe that the effect is larger at palladium than at the other metals since palladium is less noble than gold or platinum, so that palladium has a greater susceptibility to anodic oxidation. The delay in the open-circuit decay trace of e.m.f. as a function of time is found to depend on the time of previous anodic polarization, as also observed with the delay in visible gas evolution on open-circuit. The delay in the e.m.f. decay curve, in fact, is directly related to the delay in the open circuit gas evolution as shown in Fig. 2 ; both effects appear to be related to the thickness of the anodically formed layer. Less spectacular self-discharge effects with non-delayed evolution of oxygen occurring over much longer periods of time are known in oxide electrode systems and have been treated in detail in some of our previous papers[2-4]. Visible delayed gas evolution effects are not observed when the true Kolbe coupling reaction is proceeding, for example, in the electrolysis of trifluoroacetic acid, although galvanostatic charging curves again indicate formation of surface films but of thicknesses much less than those found at palladium in the formic acid solutions.

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Dielectric and Dipolar Interaction Theories of Solvent-induced Infra-red Frequency Shifts

CONSIDERABLE attention is at present being focused on the effect of solvent environment on infra-red frequencies[1-3]. The early dielectric constant treatment of Kirkwood, Bauer and Magat[4] (KBM), long known to be inadequate for the group frequencies which had been studied in any detail, namely, N—H (ref. 5) and C=O (ref. 6), has been followed by the dipolar association theory of Bellamy, Hallam and Williams[1] (BHW). They have shown that the solvent shift of a range of *X*—H stretching frequencies (where *X* = boron, carbon, nitrogen, oxygen, sulphur, halogens) can be fully accounted for in terms of solute–solvent dipolar interactions and that the bulk dielectric constant of the medium plays an insignificant part. The same theory is also able to explain the major part of the shifts exhibited by *X*=O chromophores[2]. Other factors must, however, have some influence on these dipoles as the slopes of the BHW plots obtained on plotting the relative shifts against a common standard[2] do not parallel other measures of bond polarity as would be predicted on the basis of the dipolar association theory and as has been demonstrated for *X*—H dipoles[1], in particular, N—H (ref. 7). Buckingham's elegant extension of the dielectric theory has been tested and extended by Caldow and Thompson[8] in their detailed evaluation of carbonyl and cyanide shifts. They suggest that both dielectric and specific interaction effects operate, and, by considering interaction terms, based on values of Taft's σ* inductive factors, conclude that the dielectric factor contributes about 30 per cent of the total Δν exhibited by C=O dipoles. Norrish[9] has approached the problem via solute–solution interaction energies in terms of the internal latent heats of solute and solvent and the heat of mixing. However, as he points out, lack of data does not permit a rigorous test of his relationship.

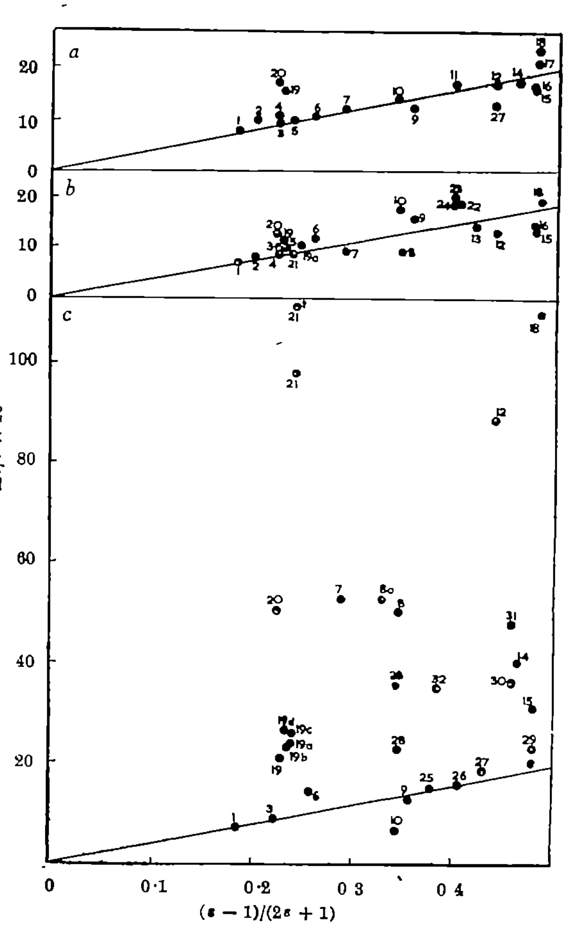

Fig. 1. Relative frequency shift at *a*, ν C—Cl (asym.) of *cis*-dichloroethylene, *b*, ν C=O of acetophenone; *c*, ν N—H of pyrrole in dilute solution as a function of the dielectric constant of the solvent

Solvents: 1, *n*-Hexane; 2, cyclohexane; 3, carbon tetrachloride; 4, cyclohexene; 5, tetrachloroethylene; 6, carbon disulphide; 7, di-*n*-butyl ether; 8, diethyl ether; 8*a*, *iso*propyl ether; 9, chloroform; 10 bromoform; 11, methyl iodide; 12, pyridine; 13, methylene chloride; 14, acetone; 15, acetonitrile; 16, nitromethane; 17, dimethyl formamide; 18, dimethyl sulphoxide; 19, benzene; 19*a*, toluene; 19*b*, *m*-xylene; 19*c*, ψ-cumene; 19*d*, mesitylene; 20, dioxan; 21, triethylamine; 22, pyrrole; 23, aniline; 24, methylene iodide; 25, chlorobenzene; 26, *sym*-tetrachloroethane; 27, 1 : 2 dichloroethane; 28, anisole; 29, nitrobenzene; 30, acetophenone; 31, cyclohexanone; 32, ethyl acetate

We are at present examining the effect of solvent environment on C—halogen frequencies. In general, as might be anticipated for a dipole the end of which most readily available for association is of the same sign, these follow the same overall pattern of solvent behaviour as that exhibited by C=O. The relative shift is also of the same order as for C=O, but a theoretical breakdown of this into the various contributions leads us to the conclusion that with these dipoles the dipolar interaction and dielectric factors play almost equal parts. The most striking outcome is the reasonable success of the KBM treatment, a typical example being shown in Fig. 1*a*, which also includes typical examples of an N—H frequency (Fig. 1*c*), taken from the data of Josien *et al.*[5], and extended by us[1], and a carbonyl frequency (Fig. 1*b*) plotted from the data of Bellamy *et al.*[2], with some additional points from these laboratories. It is immediately apparent that C—halogen KBM plots represent the most satisfactory plots to date of this function. Our analysis in terms of Buckingham's theory[8] as extended by Caldow and Thompson[3] enables us to account for the deviations from the KBM relationship which are found with highly polar solvents such as dimethyl formamide and dimethyl sulphoxide, in terms of specific dipolar interactions.

It is clear that in the development of theories of solvent effects it must be appreciated that neither of the two major factors which contributes to infra-red solvent shifts can be ignored, and that their relative significance varies from case to case, being dependent as much on the particular dipole absorber as on the solvent medium itself.

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A New Synthetic Route to Dialkylphosphinous Chlorides

PREPARATIVE methods for dialkylphosphinous chlorides are few in number, and frequently give unsatisfactory yields or require techniques unsuitable for such problems as the synthesis of radioactively labelled compounds of high activity[1-3]. A recently described reaction for the preparation of alkylphosphonous dichlorides[4] has been shown to be adaptable to the preparation of dialkylphosphinous chloride in a single-flask reaction with good yield.

It has been found that alkylphosphonous dichlorides will form a complex with aluminium chloride and an alkyl halide in the same manner as does phosphorus trichloride[5]. Solution of this complex in an electron-donating solvent, for example diethyl phthalate (DEP), followed by reduction with powdered antimony or, preferably, powdered zinc, gives good yields of dialkylphosphinous chloride on distillation.

$$\begin{tikzcd} RCl + R'PCl_2 + AlCl_3 \arrow[r] & {[RR'PCl_2]^+[AlCl_4]^-} \arrow[d, "\text{DEP}"', "\text{Zn or Sb}"] \\ & RR'PCl + [AlCl_3][DEP] + ZnCl_2 \text{ or } SbCl_3 \end{tikzcd}$$

The mixed and symmetrical products prepared by this route are given in Table 1.

Table 1

Compound	b.p /mm.	n^{t}_{d}	Yield (per cent)
$(CH_3)_2PCl$	70/690	—	45
$(i\text{-}C_3H_7)_2PCl$	69/33	1·4745$^{(27)}$	68
$(C_6H_{11})_2PCl$	92/4	1 4771$^{(23)}$	46
$(CH_3)(ClCH_2)PCl$	67/98	1·5140$^{(25 \cdot 4)}$	78
$(CH_3)(Cl_2CH)PCl$	90/100	—	41
$(CH_3)(i\text{-}C_3H_7)PCl$	82–85/208	—	66
$(ClCH_2)_2PCl$	76/22	—	22

Full details will be reported elsewhere.

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BIOCHEMISTRY

Uptake of Deoxyribonucleic Acid (DNA): a Special Property of the Cell Nucleus

ACCORDING to the concept of metabolic stability, DNA is synthesized only before cell division and during the formation of polyploid nuclei. Thus the incorporation of labelled precursors is generally considered to be a measure of DNA synthesis and, consequently, of proliferative activity of the cell population in question.

In the light of recent findings, this assumption does not have absolute validity. Pelc has observed incorporation of adenine-^{14}C and ^{3}H-thymidine into the epithelium of the vesica seminalis in amounts exceeding by 20–80 times the DNA value required for cell division[8,9]. He interprets his findings as metabolic activity of DNA connected with some cellular functions of non-growing differentiated epithelium[10]. In the case of phosphorus, however, no exchange was found in the DNA of single non-dividing cells[5,6].

I have observed DNA labelling in resting cells of bone marrow, thymus and spleen after transfusion of homologous lymphocytes labelled with phosphorus-32. Control experiments using phosphorus-32 as inorganic phosphate were mostly negative, and thus the possibility of metabolic exchange of phosphorus-32 in the DNA molecule itself was excluded[4]. It is therefore assumed that the resting cells of the recipient took up the whole DNA-phosphorus-32 or a large part of it from nuclear debris of injected donor cells.

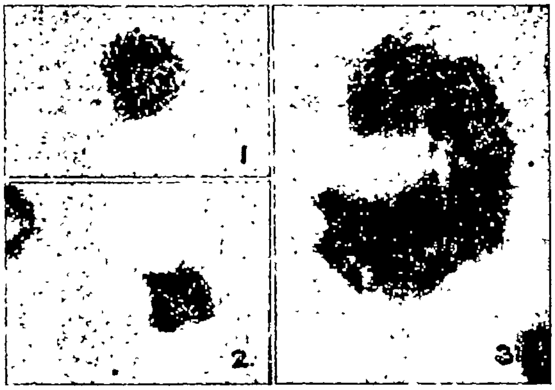

Fig. 1. Autoradiographs of the recipient's bone marrow cells 2 hr. after the application of lymphocytes labelled with phosphorus-32, showing labelling of (1) ring-shaped nucleus of a granulocyte; (2) one segment of the granulocyte nucleus; (3) a megakaryocyte in which the label occurs only in one half of the nucleus. Feulgen, squash. (× 1,725)

The distribution of the label in resting nuclei was examined in greater detail, and the preliminary results are given here.

Homologous lymphocytes labelled with phosphorus-32 were obtained from the thymuses of male *H* mice injected with 25 μc. of inorganic phosphorus-32, 21 hr. before being killed. The thymuses were suspended in isotonic Hanks's solution, the cell suspension was washed and injected intravenously into 10 males of the same mouse strain 24 hr. after total-body irradiation with a dose of 800 r.; X-rays were delivered at the rate of 60 r./min. (180 kV., 15 m.amp., filters 1 mm. aluminium and 0·5 mm. copper, target distance 45 cm.). Each irradiated mouse was given 0·1 mgm. heparin intraperitoneally and 1 ml. of suspension containing 50 million cells with a total radioactivity of 0·01 μc. per ml. The recipients were killed 2 and 6 hr. after the administration of cells. Their bone marrow fixed with acetic alcohol and stained with Feulgen was squashed in accordance with Sachs's method[11]. The non-DNA phosphorus was removed from the preparations by Feulgen acid hydrolysis[6]. The specimens were covered with liquid '*A*-2' emulsion, exposed for 1 month and developed in *D*19.

Labelled cells were found in the bone marrow of the recipients singly and in groups. The Feulgen reaction made possible the differentiation of some cell types only: granulocytes, myelocytes, reticular cells, megakaryocytes, and mononuclear cells of the lymphocyte or erythroblast series. The label occurred in the nuclei of all the types of cell mentioned. In the case of nuclei of small mononuclear cells, the grains of emulsion were evenly distributed. In the granulocytes the distribution of grains was convincing enough to prove radioactivity in the nucleus but not in the cytoplasm (Fig. 1). In some granulocytes only one of the nuclear segments was labelled (Fig. 2). The megakaryocytes often took up the label only in one part of their nucleus (Fig. 3). In the nuclei of the reticular cells, most of the grains were localized above the heterochromatin associated with the nucleolus.

The presence of DNA-phosphorus-32 in the nuclei of granulocytes, which are non-dividing differentiated cells, as well as the accumulation of label only in certain areas of the nuclei of megakaryocytes or reticular cells, exclude the possibility of phosphorus-32 in the form of simpler precursors having been utilized in DNA synthesis and/or in metabolic turnover. The distribution of label in the nuclei gives sufficient evidence that a whole DNA molecule or large portions of it penetrate through certain places on the nuclear surface. Its entrance is probably facilitated by the same mechanisms that are responsible for the penetration of cytoplasmic material giving rise to intranuclear inclusions[7]. The ensuing process of incorporating the DNA which has penetrated into certain nuclear structures remains open to further investigation. In the heterochromatin regions of reticular cells, the label persisted for 48 hr. after the administration of labelled cells[4].

Although it is not possible at present to decide whether incorporated homologous DNA performs its proper biological function, this seems to be the case with autologous DNA. The property of the cell nucleus of taking up DNA from the intercellular environment supports the hypothesis that the lymphocyte nucleic acids are re-utilized by originating cells of the ensuing lymphocyte generation. This has been hitherto based only on indirect biochemical data[1] and cytological findings in lymph node cultures[12] and in spleen lymphoid tissue[2,3].

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Prevention of Fatty Liver due to Threonine Deficiency by Moderate Caloric Restriction

Rats fed a diet containing 9 per cent casein supplemented with sulphur-containing amino-acids and choline develop moderately fatty livers, but if the diet is supplemented with threonine, the accumulation of fat is reduced[1,2]. Evidence that fat synthesis is accelerated in rats fed the threonine-deficient diet was obtained by measuring the incorporation of acetate-1-^{14}C into liver and carcass fat[3]. This suggested that rats fed this low protein diet consume an excess of calories in relation to their intake of balanced protein and that the extra calories are used for the synthesis of fat.

The present communication describes observations made on the effect of caloric intake on the production of fatty liver caused by threonine deficiency. A moderate restriction of caloric intake prevented the development of fatty livers in rats fed the threonine-deficient diet, but an equivalent restriction of caloric intake did not prevent the accumulation of liver fat in rats fed a choline-deficient diet.

Male weanling albino rats of the Wistar strain were used. The experiments consisted of two series; in one threonine-deficient diets were fed, in the other choline-deficient diets. The composition of the experimental diets is presented in Table 1. In both series, the control groups of rats were fed the respective basal diets *ad libitum*, and food consumption

Table 1. COMPOSITION OF DIETS

	Threonine-deficient diets			Choline-deficient diets	
	Basal	70 per cent calories	50 per cent calories	Basal	70 per cent calories
Casein	90	90	90	85	85
DL-Methionine	3	3	3	—	—
Sesame oil	50	50	50	50	50
Salt mixture	40	40	40	40	40
Vitamin mixture	4 5	4·5	4·5	4·5	4·5
Choline hydrochloride	1·5	1·5	1·5	—	—
Cholesterol	—	—	—	5	5
Sucrose	815	509	305	815	515
Feed ratio*	1	0 7	0 5	1	0 7

* Relative caloric intakes when diets were fed in quantities that provided equal amounts of protein.

was measured daily. The other groups of rats in each series were fed the same amounts of protein and amino-acids as their respective control groups consumed, but the caloric intakes were 70 or 50 per cent of their respective control groups. At the end of two weeks the rats were killed, their livers were removed and fat was extracted with ether from the dried and finely powdered liver.

As shown in Table 2, the fat content of the livers of the control rats in the threonine-deficient series was 20·3 per cent on a dry weight basis whereas the value for the rats that received 30 per cent less calories was 13·3 per cent, within the normal range. Thus, a moderate reduction in caloric intake with an equal intake of protein prevented the fatty infiltration without appreciably reducing growth. Reduction of caloric intake to 50 per cent of that of the control group further decreased liver fat content (9·2 per cent) but also retarded growth. These results suggest that the balance between amino-acid and caloric intake is important in controlling liver fat accumulation.

Table 2. EFFECT OF CALORIC INTAKE ON FAT CONTENT OF THE LIVERS OF RATS FED ON THREONINE- OR CHOLINE-DEFICIENT DIETS

	Threonine deficiency			Choline deficiency	
	Basal	70 per cent calories	50 per cent calories	Basal	70 per cent calories
Gain for 2 weeks (gm.)	35·5	32 1	16 2	17 0	6 8
Protein intake for 2 weeks (gm.)	11·4	11·4	11·4	8 8	8 8
Caloric intake for 2 weeks	518	363	259	427	299
Liver fat (per cent*)	20·3 ± 3 2†	13 3 ± 0·3	9·2 ± 0 2	30·1 ± 3·6	28·6 ± 4 0

* Liver fat is expressed as percentage of dry liver.
† *S.E.*

This effect was not observed when the fatty liver was caused by choline deficiency (Table 2). The growth of the group fed the choline-deficient diet and 30 per cent less calories than the control group was markedly reduced; however, there was no significant fall in liver fat content. Best and Ridout[4] found that the fatty liver of choline deficiency was prevented by severe, but not by moderate, caloric restriction.

A reduction in caloric intake would decrease the quantity of precursors for fat synthesis. If a fatty liver were caused by an acceleration of fat synthesis due to an excessive carbohydrate intake a reduction in caloric intake would be expected to decrease the fat accumulation. However, if the fatty liver were primarily a result of impaired transport of fat, a reduction in caloric intake would not be expected to affect fat accumulation so much. In this connexion, these results are in agreement with those which indicated that there is an accelerated incorporation of acetate-1-^{14}C into liver fat in threonine-deficient rats[3].

The fatty liver caused by feeding rats a low protein diet that is primarily deficient in threonine appears to be a result of a disproportionately high intake of calories in relation to the intake of balanced protein. This emphasizes the need for further work on the relationship between protein and caloric utilization on animals and subjects having low intakes of protein.

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Polypeptide Chains of Hæmoglobin A_2

HÆMOGLOBIN A_2 (Hb-A_2), first described by Kunkel and Wallenius[1], is a minor component found in normal adult hæmolysate which has an electrophoretic mobility slower than Hb-A at alkaline pH. Several variants of Hb-A_2 have been described and these now fall into two groups. While the minor hæmoglobins of one group can occur when Hb-A is normal, for example, Hb-A'_2 (ref. 2) and Hb-B_2 (ref. 3), those of the other group are always associated with α-chain variants of Hb-A. Shooter, Skinner, Garlick and Barnicot[4] described a minor hæmoglobin variant of the latter group which they called Hb-G_2 in an individual carrying Hb-G_{Ibadan}. Since the charge relationship between Hb-G_2 and Hb-$G_{\text{Ib.}}$ was similar to that between Hb-A_2 and Hb-A, Gammack, Huehns, Shooter and Gerald[5] suggested that Hb-G_2 was the naturally occurring hybrid of Hb-A_2 and Hb-$G_{\text{Ib.}}$ This hypothesis implied not only that Hb-$G_{\text{Ib.}}$ has abnormal α_2 sub-units, but also that Hb-A_2 differs from Hb-A only in the composition of the non-α_2 sub-unit and has the molecular formula $\alpha_2^A\delta_2^{A_2}$. It has already been shown[5] that Hb-$G_{\text{Ib.}}$ consists of normal β_2^A and abnormal α_2^G sub-units, and the results now reported are consistent both with the above formula for Hb-A_2 and with the suggestion that Hb-G_2 carries the abnormal sub-unit of Hb-$G_{\text{Ib.}}$ and the specific sub-unit of Hb-A_2.

Hybridization experiments were carried out using a microscale adaptation[5] of the original recombination method developed by Itano and Robinson[6]. Whereas most variants of Hb-A give satisfactory hybrid formation after exposure to pH 4·7 for 4 hr.[7] no new hæmoglobin species could be detected in similar experiments with Hb-A_2. However, if the time of dialysis against acetate buffer at pH 4·7 was extended to 72 hr. or more, amounts of hybrid hæmoglobins sufficient for their detection also formed with Hb-A_2. Thus, when mixtures of the isolated hæmoglobins Hb-A_2 and Hb-G_a were dissociated for these longer

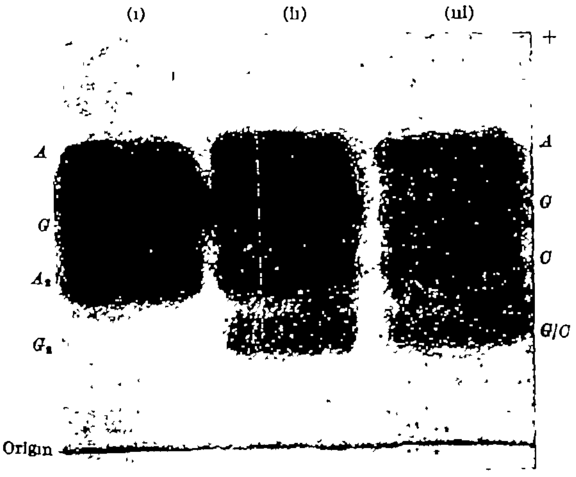

Fig. 1. Hybridization of hæmoglobins A_2 and G_{Br} Starch-gel analysis in the discontinuous system. Migration time 2 hr., *o*-dianisidine stain. (i) Hb-A_2 + Hb-$G_{Br.}$ untreated mixture; (ii) Hb-A_2 + Hb-G_{Br} dissociated at *p*H 4·7 for 96 hr. and then recombined; (iii) Hb-*A*, $G_{Br.}$, *C*, *G*/*C* marker

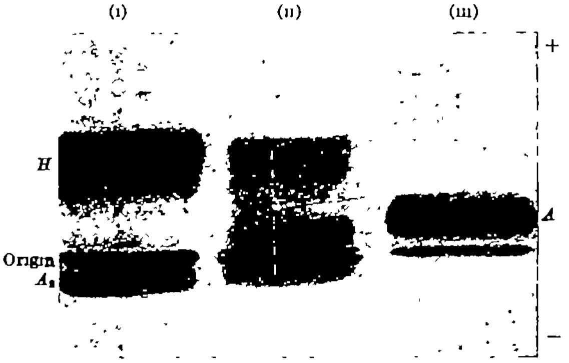

Fig. 2. Hybridization of hæmoglobins A_2 and *H*. Starch-gel analysis in phosphate buffer, gel *p*H 7 4. Migration time 2 hr., *o*-dianisidine stain. (i) Hb-A_2 + Hb-*H* untreated control mixture; (ii) Hb-A_2 + Hb-*H* dissociated for 72 hr. and then recombined; (iii) Hb-*A* marker
(The movement of the new zone (Hb-*A*) in (ii) is slightly decreased by the more concentrated Hb-A_2 zone migrating towards the opposite electrode when compared with the Hb-*A* marker (iii))

periods and then recombined at *p*H 6·8, a new hæmoglobin zone migrating more slowly than Hb-A_2 and in the position of Hb-*G*/*C* appeared and some intensification of the Hb-*A* zone occurred (Fig. 1). With isolated Hb-*H*, Hb-A_2 gave Hb-*A* (Fig. 2). No new species appeared when Hb-A_2, Hb-G_a or Hb-*H* by themselves or a mixture of Hb-A_2 and Hb-*S* were dissociated and recombined in parallel experiments.

The formation of Hb-*A* in the hybridization with Hb-*H* suggests that Hb-A_2 has α_2^A sub-units similar to those found in Hb-*A*.

$$\underset{\text{Hb-}A_2}{\alpha_2^A\delta_2^{A_2}} + \underset{\text{Hb-}H}{\beta_4^A} \rightarrow \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A} + \underset{\text{Hb-}A_2}{\alpha_2^A\delta_2^{A_2}} + (\beta_2^A + \delta_2^{A_2})$$

(The fate of the sub-units enclosed in brackets has not been determined.)

The charge difference between Hb-A_2 and the new hæmoglobin species formed in the dissociation and recombination experiment with Hb-$G_{Br.}$ is similar to that between Hb-*C* and Hb-*G*/*C*. The structural difference between these two is the replacement of the α_2^A sub-unit of Hb-*C* with an α_2^G sub-unit[6]. It therefore follows that the difference between Hb-A_2 and the new hæmoglobin formed in the hydridization experiment with Hb-$G_{Br.}$ could also be the replacement of an α_2^A sub-unit in Hb-A_2 by an α_2^G sub-unit. The reaction may then be written as follows:

$$\underset{\text{Hb-}G}{\alpha_2^G\beta_2^A} + \underset{\text{Hb-}A_2}{\alpha_2^A\delta_2^{A_2}} \longrightarrow \underset{\text{Hb-}A}{\alpha_2^A\beta_2^A} + \underset{\text{Hb-}G}{\alpha_2^G\beta_2^A} + \underset{\text{Hb-}A_2}{\alpha_2^A\delta_2^{A_2}} + \underset{\text{Hb-}G_2}{\alpha_2^G\delta_2^{A_2}}$$

Charge difference
from Hb-*A*:

+ 2 + 4 0 + 2 + 4 + 6

These hybridization experiments are, therefore, consistent with the hypothesis that Hb-A_2 has the general formula $\alpha_2^A\delta_2^{A_2}$.

Raper, Gammack, Huehns and Shooter[8] have suggested from the study of two individuals carrying the four hæmoglobins *A*, $G_{Br.}$, *C* and $G_{Br.}/C$ that the last stage of hæmoglobin synthesis is the combination of α_2 with β_2 sub-units. If this also applies to Hb-A_2, in this instance α_2^A sub-units combining with $\delta_2^{A_2}$ sub-units, then the α_2^A sub-units for the formation of Hb-*A* and Hb-A_2 could be derived from a common pool. It would then follow that an individual who made α_2 sub-units different from those found in Hb-*A* would not only show the corresponding variant of Hb-*A* but also of Hb-A_2. This has been found to be so. In the individual with Hb-*G* disease, who only makes α_2^G sub-units, no Hb-A_2 but a different minor component, Hb-G_2 ($\alpha_2^G\delta_2^{A_2}$), was found[4,5]. Both Hb-A_2 and Hb-G_2 were seen in two unrelated individuals carrying Hb-*A* as well as Hb-G_a (unpublished observations).

It also follows that if the α_2^A sub-units of both Hb-*A* and Hb-A_2 are derived from a common pool, then their formation is controlled at the same genetic locus.

The formation of the other series of Hb-A_2 variants which can occur without any concomitant abnormality of Hb-*A*, such as Hb-A_2' (ref. 2) and Hb-B_2 (ref. 3), indicates that there is a further, separate locus controlling the synthesis of Hb-A_2 and it is suggested that this locus, the δ locus, determines the formation of the $\delta_2^{A_2}$ sub-units. The genotype relevant to the formation of Hb-A_2 in a normal individual would then be α^A/α^A, $\delta^{A_2}/\delta^{A_2}$.

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Composition of Various Ribonucleic Acid Fractions from Micro-organisms of Different Deoxyribonucleic Acid Composition

THE ratio of guanine plus cytosine to adenine plus thymine (GC/AT) in the deoxyribonucleic acid (DNA) of a given species of a micro-organism is constant, but the GC/AT ratio of different kinds of micro-organisms can vary by a factor of as much as five times[1]. The ratio of GC content to AU (uracil) content of cell ribonucleic acid (RNA) varies only slightly from one organism to another; but this ratio tends to be slightly higher in organisms the DNA ratio of which is high[2]. These facts raise a question as to whether all the RNA of a cell has the same composition or whether perhaps a small RNA sub-fraction has the same base ratios as its corresponding DNA (uracil being equivalent to thymine). Recent experiments have demonstrated an RNA sub-fraction in yeast which does have a composition almost identical to the corresponding DNA (ref. 3). The present work is concerned with analysing the nucleotide ratios in sub-fractions of the RNA from organisms with varying GC/AT ratios in the DNA.

The organisms chosen for this work were *Diplococcus pneumoniae*, in which GC is equal to 38 per cent of total DNA bases; *Escherichia coli*, GC of 50 per cent; and *Micrococcus lysodeikticus*, GC of 72 per cent (ref. 4 and Guild, W. R., personal communication). The organisms were grown in 'Trypticase-soy' medium (Balt. Biol.) at 37° C., with the exception of *D. pneumoniae*, grown in 'Bactotryptose' medium (Difco)[5]. The bacterial growth was collected by centrifugation, suspended in a 0·02 *M tris*(hydroxymethyl)aminomethane buffer containing 0·01 *M* magnesium chloride, and the cells ruptured by passage through a French pressure cell at 5,000 lb. pressure. The resulting suspension was cleared of cell debris by low-speed centrifugation followed by centrifugation at 100,000*g* for 5 min. Deoxyribonuclease was then added to break down DNA to relatively small polynucleotide units. It is impossible for DNA of this size-range to produce significant contamination of any of the final RNA fractions defined here. Three RNA fractions were experimentally defined as follows; a first pellet resulted from centrifuging the cell extracts for 2 hr. at 100,000*g* in a refrigerated Spinco model *L* centrifuge. This pellet should contain all microsomal material of 50*S* sedimentation constant and larger as well as most 30*S* microsomes[6]. A second pellet resulted from centrifuging the supernatant of the previous centrifugation for 5 hr. at 100,000*g*. This should contain mainly material sedimenting with sedimentation constants between 15*S* and 30*S*. For the third fraction, the supernatant of the second fraction was adjusted to *p*H 5 with acetic acid, the resulting precipitate collected by low-speed centrifugation, and redissolved in *p*H 7 buffer. This solution contains the soluble RNA of the cell[7]. Each of the nine fractions defined above was then extracted with water-saturated phenol twice, according to Kirby[8]. Relatively pure RNA was then precipitated by addition of two volumes of cold ethanol, collected by centrifugation, washed again in ethanol, and stored at −20° C. Approximately 10 mgm. of RNA from each fraction was hydrolysed in 1 *N* potassium hydroxide at 35° C. for 18 hr., the potassium ion being afterwards removed by neutralization with perchloric acid. This procedure degraded the RNA to a mixture of 2′- and 3′-nucleotides[9]. The ribonucleotides of the four main bases were separated by paper electrophoresis in a buffer at *p*H 3·6, according to the methods of Markham and Smith[10]. The paper strips containing the nucleotides were afterwards scanned with an ultra-violet lamp ('Mineralight^R') to locate the separate nucleotide bands. Each band was then cut out and eluted overnight in phosphate buffer *p*H 7·5. The ultra-violet absorption spectrum for each nucleotide band was determined in the range 2350–2900 Å. Known mixtures of 2′-, 3′-ribonucleotides were run through the entire hydrolysis procedure in order to determine properly the amounts of each nucleotide present in the RNA hydrolysates. 7–13 electrophoretic runs were made on each fraction. At least two separate hydrolyses by potassium hydroxide of each microsomal RNA fraction occurred. No significant differences in nucleotide ratios between different hydrolyses of the same RNA fraction were observed.

Table 1. NUCLEOTIDE COMPOSITION OF RNA FRACTIONS. *C*, *G*, *U* AND *A* REFER TO CYTIDYLIC, GUANYLIC, URIDYLIC AND ADENYLIC ACIDS RESPECTIVELY

		Large microsome RNA	Small microsome RNA	Soluble RNA
	C	19·2 ± 0·6	18·9 ± 0·5	22·9 ± 0·5
	G	31·1 ± 1·0	30·7 ± 0·7	29·7 ± 0·8
D. pneumoniae	*U*	24·0 ± 0·7	24·8 ± 0·5	25·0 ± 0·3
	A	25·4 ± 0·4	25·4 ± 0·6	22·2 ± 0·2
	C + *G*	50·3 ± 1·6	49·6 ± 1·2	52·6 ± 1·3
	U + *A*	49·4 ± 1·1	50·2 ± 1·1	47·2 ± 0·5
	C	20·1 ± 0·8	20·7 ± 0·8	24·7 ± 0·5
	G	32·4 ± 0·8	31·7 ± 0·7	32·0 ± 0·9
E. coli	*U*	22·6 ± 0·4	23·2 ± 0·5	22·5 ± 0·4
	A	24·7 ± 0·3	24·2 ± 0·4	20·5 ± 0·5
	C + *G*	52·5 ± 1·6	52·4 ± 1·0	56·7 ± 1·4
	U + *A*	47·3 ± 1·9	47·6 ± 0·9	43·0 ± 0·9
	C	20·8 ± 0·4	22·1 ± 0·5	25·1 ± 0·5
	G	33·3 ± 0·8	31·5 ± 1·3	31·7 ± 0·8
M. lysodeikticus	*U*	22·7 ± 0·7	24·1 ± 0·9	23·4 ± 0·5
	A	23·0 ± 0·5	22·2 ± 0·8	19·6 ± 0·5
	C + *G*	54·1 ± 1·2	53·6 ± 1·8	56·8 ± 1·3
	U + *A*	45·7 ± 1·2	46·3 ± 1·2	43·0 ± 1·0

The results are presented in Table 1. The values represent averages of all determinations on a given sample. Errors are presented as probable error. The base ratios for the first RNA fraction of *E. coli* (large microsomes) are the same as those given by Spahr and Tissières for *E. coli* 70*S* microsomes, except for the value of cytosine, which is significantly lower in the present instance[11].

The most obvious result in Table 1 is that none of these RNA fractions has a base ratio approaching that of cell DNA. However, the DNA composition of a given organism does seem to be reflected slightly in the composition of all the RNA fractions. In all three such fractions from each organism there is a statistically significant increase in cytosine and decrease in adenine as the GC content of cell DNA increases. In two of the three cases there is also a significant rise in guanine content, but the results do not give a clear indication of uracil changes as a function of GC content of DNA; a drop in uracil is suggested.

Generally, there is no statistically significant difference between the composition of RNA from small microsomes and that of RNA from larger ones for any given organism. The only exception to this is that in *M. lysodeikticus* cytosine represents a significantly higher per cent of total bases in small microsomes than in large ones. Spahr and Tissières found similarly that the fraction of cytosine in RNA from 30*S* microsomes was significantly higher than that in 50*S* microsomal RNA in *E. coli*[11].

A difference in composition between soluble RNA and microsomal RNA exists. Soluble RNA is significantly higher in cytosine and lower in adenine than is microsomal RNA. However, there is no significant difference in the guanine and uracil composition of the microsomal and soluble RNA's.

In these experiments I have been unable to demonstrate a fraction of cell RNA that has a composition equal to that of its corresponding DNA (assuming uracil to be equivalent to thymine). However, as stated above, such a fraction has been reported for yeast. It seems a possibility then, that all the fractions in the present work are heterogeneous in that they contain a small amount of RNA which has base ratios equivalent to its corresponding DNA, thus producing slight variations in the average base ratios of each fraction in the direction of the base ratios of the corresponding DNA's.

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Total Cytochrome Oxidase Activity and its Relation to Basal and Maximal Metabolism

THE study of the values of the basal metabolic rate in mammals and its relation to body-weight has been the object of many papers and reviews since the middle of the nineteenth century. On the other hand, very few data are available concerning the upper limit of animal metabolism; only individual values are found in the literature concerning the maximal metabolism of some mammals and man. The incompleteness of the available data and their suitability for comparative purposes are connected with the difficulty in obtaining the state of maximal performance in animals. In addition, in many of the animals used, maximal oxygen consumption can be obtained only under simultaneous application of work and lowered temperatures, so that the working and thermoregulatory capacity of the animals are used as well. In these conditions, however, a rapid decrease of metabolism occurs as a result of cooling the animals, as I have already shown[1]; it is very difficult, therefore, to find out the period of greatest oxygen consumption. Nearly all the data in the literature seem to be affected by this error, and the results obtained are, therefore, probably lower than true maximal values.

I have looked for other possibilities for a more precise determination of maximal metabolism. It has been found that there exists a constant quantitative relationship among different oxidative enzymes and that the cytochrome oxidase activity readily becomes adapted to various conditions; this suggests that an excess of oxidative enzymes is not present in tissues. It would be expected, therefore, that the maximal possible oxygen consumption of animals and organs will correspond to the terminal oxidative enzyme, the cytochrome oxidase.

I have therefore determined the cytochrome oxidase activity of whole organisms at 37° C. manometrically[3], and the values of oxygen consumption thus obtained have been compared with the values of maximal working metabolism measured in living animals. Before homogenization the animals were depilated, the digestive tract removed and the carcase ground in a meat grinder.

The total cytochrome oxidase activity was determined in six species of rodents (bank vole, field vole, mouse, golden hamster, rat, guinea pig) of body-weight 19–700 gm. It was found that the total cytochrome oxidase activity is proportional to a power of the body-weight, the exponent being very near that found for basal metabolism, namely, 0·67 (Table 1). A similar relationship was also found in various organs and in the musculature of some other mammals[4].

Table 1. AVERAGE CYTOCHROME OXIDASE ACTIVITY IN VARIOUS MAMMALS

Species	Weight of animals without digestive tract (gm.)	Weight of dry substance (gm.)	Cyt. ox. activity (mm.^{3} oxygen/hr./mgm. dry substance)	Total cyt ox. activity (ml. oxygen)	Month
Guinea pig	521·0	202 0	24 1±6·4	4,870	V–VI
Rat	254 0	70 0	24 1±1 7	1,690	III–VI
Golden hamster	64 0	16 8	62 6±3 6	1,050	IX
Mouse	20 6	5 0	62·9±6 7	316	III–IV
Bank vole	17 5	5 4	60 5±8 1	327	IX–XI
Field vole	16·1	5 2	63·0±8·0	327	X

The absolute values of the total cytochrome oxidase activity are found to be very nearly the same as the maximal metabolism of whole animals; the difference does not exceed ± 25 per cent (Table 2). Later experiments have shown that the values of cytochrome oxidase activity measured at 37° C. are not the true maximal values; maximal oxygen consumption is reached at other temperatures, which are different for different animals[4]; this factor may diminish the difference between the values of total cytochrome oxidase and maximum metabolism.

Table 2. COMPARISON OF MAXIMAL METABOLISM VALUES WITH VALUES OF AEROBIC METABOLIC CAPACITY IN VARIOUS MAMMALS

Species	Maximal metabolism (ml. oxygen/hr./gm.)	Cyt. ox. activity (ml. oxygen/hr./gm.)*	Difference (percentage of maximal metabolism)
Golden hamster	13·40	16,40	+ 22
Mouse	17 50	15 35	– 12
Field vole	16 10	20 30	+26
Bank vole	22·40	18·60	– 17

* Cytochrome oxidase activity was measured at 37° C. and referred to 1 gm. of body-weight without digestive tract.

A similar close correspondence between the values of the total cytochrome oxidase activity and maximum oxygen consumption during physical work has been recently found in an insect[6]. Thus this seems to indicate a more general relationship. The methods with which these two values were obtained are, however, so different that they should not be regarded as physiologically identical, and I suggest the term 'aerobic metabolic capacity' for the total activity of cytochrome oxidase, measured manometrically at the optimum temperature and expressed as oxygen consumption, while the values obtained with living animals should be referred to as 'maximal metabolism'.

It seems to me that the determination of the aerobic metabolic capacity may be of value, especially for comparative purposes, as it is readily found and is much more reproducible than the value of maximal metabolism.

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A Requirement for 2-Thiouracil of 2-Thiouracil-grown *Escherichia coli* under Conditions of Ornithine Transcarbamylase Repression

PYRIMIDINE analogues may interfere with nucleic acid metabolism by inhibition of the conversion of the free pyrimidine bases to the corresponding nucleotides or by production of abnormal nucleic acids containing substituted pyrimidines. Recently it has been found that an analogue may also function by negative feed-back control and thus inhibit the formation of the normal metabolite[1,2].

This communication describes an additional effect of the uracil analogue 2-thiouracil which was observed under conditions of ornithine transcarbamylase repression. A mutant requiring ornithine, citrulline or arginine (*M*-39 *A*-23) derived from *Escherichia coli* strain *W* was used. 2-Thiouracil was added to cells of this mutant, in logarithmic phase, grown with aeration in minimal medium supplemented with glucose and arginine. As is shown in Fig. 1, a linear increase in optical density with time from 45 to 180 min. is observed. When cells grown for 45–135 min. in the presence of 2-thiouracil were plated on minimal medium supplemented with arginine, up to 90 per cent of the cells lost their ability to form colonies (Fig. 2).

However, when the 2-thiouracil-grown cells were plated on minimal medium containing 2-thiouracil, all the cells were capable of colony formation. It can be seen from Fig. 2 that after pre-incubation periods with 2-thiouracil exceeding 135 min., the proportion of the cell population capable of colony formation on minimal medium without 2-thiouracil increased. Eventually the same colony count was observed on minimal agar plates with or without 2-thiouracil. The transitory nature of the 2-thiouracil

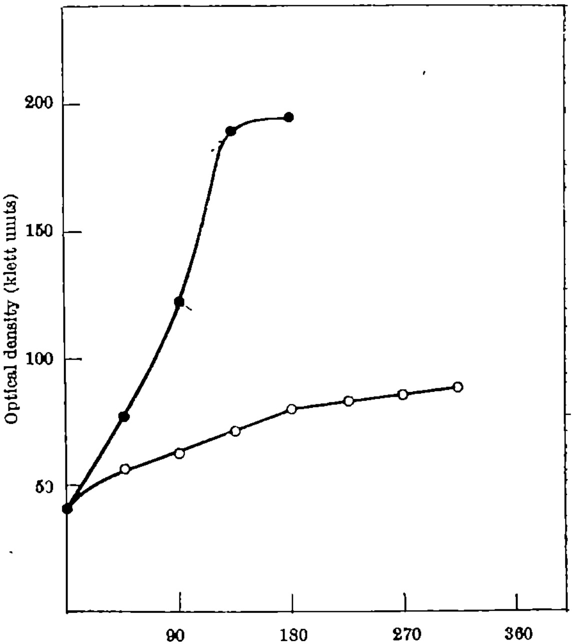


Fig. 1. Growth curve of strain *M*-39 *A*-23 growing in the presence of 20 μgm./ml. arginine with (○) and without (●) 200 μgm./ml. 2-thiouracil

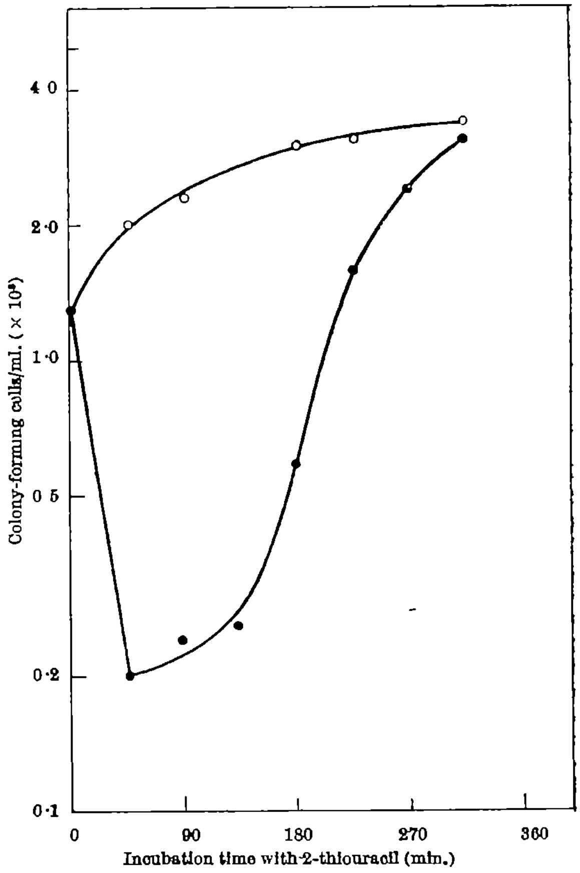


Fig. 2. Colony counts of strain *M*-39 *A*-23 preincubated with 20 μgm./ml. arginine and 200 μgm./ml. 2-thiouracil for the periods indicated, then plated on minimal agar plates with (○) and without (●) 50 μgm./ml. 2-thiouracil

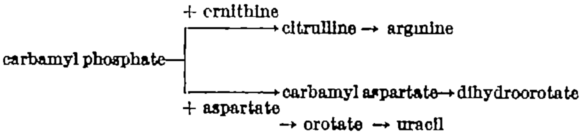
requirement was found to be due to changes in the level of arginine in the pre-incubation medium. Exhaustion of arginine relieved the cells from the 2-thiouracil requirement.

The 2-thiouracil requirement was also obtained when the mutant was grown with 2-thiouracil in the presence of citrulline. However, when the mutant was grown with 2-thiouracil in the presence of ornithine, inhibition of growth was less marked and neither loss of viability nor a 2-thiouracil requirement was observed when these cells were plated on to minimal medium.

Wild-type *E. coli W* cells are only slightly inhibited by 2-thiouracil. However, when arginine was added to the growth medium, 2-thiouracil exerted a marked inhibitory effect and also showed the 2-thiouracil requirement.

In all the experiments described, the 2-thiouracil requirement occurred when the synthesis of ornithine transcarbamylase, which catalyses the conversion of ornithine to citrulline, was repressed. This repression occurs in *E. coli W* and its mutant with arginine or citrulline but not with ornithine. In *E. coli B*, a non-repressible strain[3], no 2-thiouracil requirement was observed under the conditions described.

These results suggest a mechanism based on the metabolic relationship existing between arginine and uracil :



It has been suggested that these syntheses are to some extent competitive[4] and regulated by negative feed-back mechanisms[4,5].

Preliminary results show that 2-thiouracil mimics the feed-back action of uracil. Experiments are now in progress to elucidate the mechanism of the 2-thiouracil effect described.

We are indebted to Dr. B. D. Davis for strain *M*-39 *A*-23 used in this investigation.

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A Fatty Acid / Protein Complex in Human Hair

In an earlier publication[1] a new sensitive method for the detection of small amounts of damage to human hair was reported. The method involves counting the numbers of cuticular and cortical cells in the liquid after treating the hair under standard conditions with papain/bisulphite solution. When the ratio of cuticle cells to cortical cells is low, the hair is assumed to have been damaged in some way. Soxhlet extraction of the hair with ethanol increases the rate of breakdown, but ether, benzene and many other non-polar solvents do not affect the ratio. It was suggested that a protective substance soluble in alcohol but not in non-polar solvents exists in the hair which maintains it intact during papain digestion. This protective substance has now been isolated from ethanol extracts and identified as a fatty acid/protein complex.

Isolation by solvent fractionation was impracticable because the materials denatured at liquid/liquid interfaces. Silica fractionation by the method of Hanahan[2] was also unsatisfactory as the material was irreversibly adsorbed on the silica. Successful isolation was achieved by electrophoresis on polyurethane sponge in dilute acetic acid at *p*H 2·5. Under these conditions fatty acids and neutral fatty materials do not move, but the complex moves towards the negative electrode. Analysis of the isolated complex shows that it consists of 20–30 per cent fatty acid and 60–70 per cent protein, which yield substantial amounts of lysine but very little cystine. Methyl esters of the fatty acids are not volatile under normal GLC conditions, but the mass spectrometer indicates there is considerable variation between samples. Treatment of alcohol-extracted hair with an alcoholic solution of the complex reduces the rate of breakdown of hair to the normal level. This is not due to enzyme inactivation, since the degradation of casein by papain is not affected by the presence of the complex. The complex is positively charged in acid solutions and the rate of migration on paper electrophoresis decreases as the *p*H is made more alkaline until no movement is observed at *p*H 6·0. The complex then remains at the origin at *p*H values up to 9·5. This, combined with the high level of lysine, suggests that the fatty acid is combined through the amino-groups of the lysine so that the effective isoelectric point is in the range for normal proteins. In alkaline solution, negatively charged fatty acid residues are removed and this maintains the electrical neutrality of the protein.

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PHYSIOLOGY

Distribution of Acetylcholine and Cholinesterase in the Heart of White Rats

THE opinion has been advanced that acetylcholine acts in the heart not only as the mediator of the vagus nerve but also as the so-called local hormone in local extraneural regulation of heart activity and that in this last function it is closely associated with heart automation[1]. If this view is correct, it can be assumed that the most rapid turnover of acetylcholine will occur in those parts of the heart which possess the highest degree of automation. In order to verify the correctness of this assumption, experiments were carried out in which the distribution of acetylcholine and cholinesterase in different parts of white rat heart was examined.

White rats of either sex were used. The animals weighed 190–365 gm. On account of the small amount of tissue available, two animals had to be used for each experiment when the content of acetylcholine was estimated and four animals when the activity of cholinesterase was assayed. The animals were killed by a blow on the head and decapitated. The heart was removed and sectioned as follows: the atria were separated from the ventricles and cut into three parts; the first part represented the region near the opening of the right superior vena cava into the right atrium, the second part the rest of the right atrium with the septum and the third part represented the left atrium; samples of suitable size were removed from the left and right ventricles. The content of acetylcholine bound in heart tissue was estimated by Rothschuh's method[2], employing modifications proposed by Vlk[3]. The activity of cholinesterase was assayed on the basis of the rate of degradation of acetylcholine during incubation of the homogenate of the corresponding part of the heart in a medium containing 200 μgm. acetylcholine chloride per ml. for 30 min. at 38° C. The concentration of acetylcholine was estimated by a chemical method based on the transfer of acetylcholine to the ferric complex of acetohydroxamic acid. Intensity of the red colour was measured with a Coleman spectrophotometer at 540 mμ.

Table 1. CONTENTS OF ACETYLCHOLINE (ACH) AND ACTIVITIES OF CHOLINESTERASE (CHE) IN DIFFERENT PARTS OF WHITE RAT HEART
The figures in brackets represent the number of experiments

Part of the heart	ACh content ± *S.D.*	Activity of ChE ± *S.D.*
The region near the opening of the right superior vena cava into the right atrium	7·07 ± 1·15 (9)	129 ± 8·74 (6)
The rest of the right atrium with septum	5·91 ± 0 94 (9)	114 ± 2·35 (5)
Left atrium	2 16 ± 0·56 (9)	103 ± 2·06 (6)
Right ventricle	—	79 ± 4·86 (6)
Left ventricle	—	71 ± 2 65 (6)

The results are summarized in Table 1. The content of acetylcholine is expressed as μgm./gm. fresh tissue and the activity of cholinesterase as μgm. acetylcholine chloride decomposed by 1 ml. mixture. The content of acetylcholine was determined in the atria only as its distribution in heart ventricles of rats had been known from the paper of Rothschuh[4]. The ventricles are known to contain much less acetylcholine than the atria, the right ventricle having a higher content of acetylcholine than the left one. The highest content of acetylcholine and activity of cholinesterase were detected in the region near the opening of the superior vena cava, whence the levels decrease in the direction of listing the individual parts of the heart in Table 1. The above-mentioned gradient of distribution of acetylcholine and cholinesterase was found in all cases investigated here. The content of acetylcholine decreases much more markedly than the activity of cholinesterase, as shown in Fig. 1, in which the content of acetylcholine and the activity of cholinesterase are expressed as percentage of the basic value which was identified with the value from the opening of the superior vena cava. The right-hand side of Fig. 1 represents the distribution of cholinesterase, the left-hand side the distribution of acetylcholine; the data of the content of acetylcholine in the right and left ventricles were computed from Rothschuh's results[4].

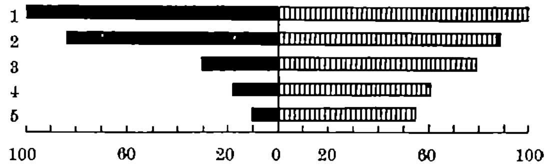

Fig. 1. The distribution of acetylcholine (black) and cholinesterase (shaded) in different parts of white rat heart. 1, Values for the region near the opening of the right superior vena cava in the right atrium; 2, the rest of the right atrium with septum; 3, the left atrium; 4, the right ventricle; 5, the left ventricle

It follows from the results that the highest content of acetylcholine and the highest activity of cholinesterase are to be found in that part of the heart where the rhythmic impulses regulating the automatic activity of the heart are normally generated. This finding is in agreement with the results of Mazel and Holland[5], who found that the most rapid turnover of acetylcholine in poikilothermic animals occurs in the sinus venosus, that is, in the part of the heart which possesses highest automation as assessed from the frequency of contractions of isolated parts. Our results support the view that acetylcholine turnover is of considerable significance in processes which represent the basis of automatic activity of the heart. A detailed report will be published in *Physiologia bohemoslovenia*.

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Pharmacological Specificities of Neurones in an Elementary Central Nervous System

RECENT pharmacological investigations by us[1] on the central nervous system of the mollusc *Aplysia depilans* revealed acetylcholine as a possible synaptic transmitter of inhibition. The evidence was obtained by electrophysiological methods; different cells in the abdominal ganglion of this animal were impaled by glass microelectrodes according to previously described techniques[2] and their reactions observed in the presence of different drugs. It was thus shown that externally applied acetylcholine in concentrations up to 10^{-12} hyperpolarizes the membrane and mimics the inhibitory postsynaptic potentials in cells having inhibitory input (conventionally designated as *H*-cells), whereas other cells having only excitatory input (*D*-cells) are depolarized by acetylcholine (Fig. 1*A*). In both cases the impedance of the membrane drops to a low level. The action of acetylcholine on *H*-cells is reversed when the level of polarization of the membrane is increased by artificially applied current. The reversal-level for acetylcholine potentials and inhibitory postsynaptic potentials have equal values, suggesting an identical selective permeability variations of the membrane. In *D*-cells the equilibrium-level is close to the zero membrane potential, indicating a different and non-selective permeability change. Local electrophoretical injections of acetylcholine disclosed a type of response which is identical in synaptic regions as well as in proximal extrasynaptic

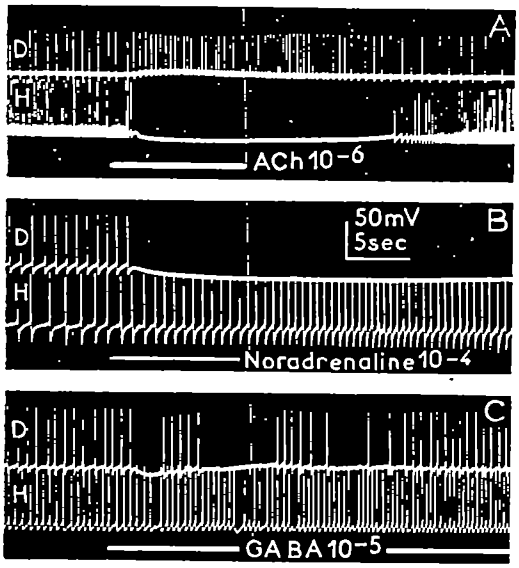

Fig. 1. Action of different drugs (horizontal line) on the spike activity of spontaneous *D* and *H Aplysia* ganglion cells. Simultaneous intracellular recordings from two neurones

membranes ; but the axonal synaptic fields are more sensitive to acetylcholine than the somatic membrane where, as usual in molluscs, there are no synaptic terminals[3]. D-Tubocurarine and atropine in external solutions reduce the inhibitory postsynaptic potentials as well as the action of acetylcholine, whereas eserine first lengthens the inhibitory effect and then reduces the size of inhibitory postsynaptic potentials by inactivating the postsynaptic membrane ; excitatory postsynaptic potentials in *H*-cells are not affected. Consequently, we concluded that the inhibitory synapse in the *Aplysia* central nervous system is cholinergic.

Very striking were the opposite reactions to acetylcholine of contiguous cells ; and it was interesting to see that this specific difference of the two types of neurones appears also with other types of drugs usually considered as having a synaptic action. However, the type of response is different from acetylcholine action. Noradrenaline in solutions up to 10^{-4}, in contrast to acetylcholine, hyperpolarizes and inhibits the *D*-neurones and activates the *H* type (Fig. 1*B*). Adrenaline has the same effect, but is about five times less active than noradrenaline.

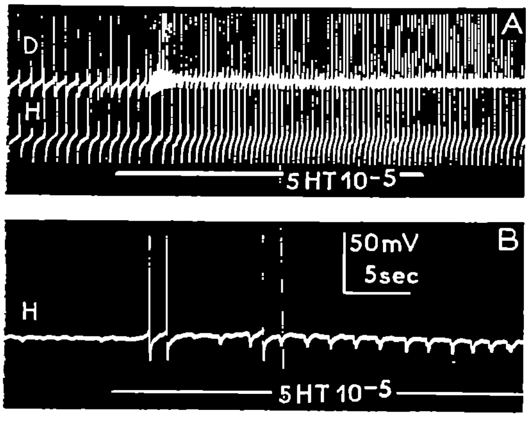

Fig. 2. *A*, Simultaneous intracellular recordings from two spontaneous *Aplysia* cells of different type on application of 5-hydroxytryptamine. *B*, The primary excitatory effect of 5HT on an *H*-cell is masked by inhibitory postsynaptic potentials due to a simultaneous activation by the drug of inhibitory interneurones

γ-Amino-butyric acid (GABA), the participation in inhibitory processes of which was recently discussed[4], usually has a depolarizing and excitatory effect upon the *H*-cells, whereas the *D*-cells, devoid of inhibitory input, are always hyperpolarized and inhibited (Fig. 1*C*). In some cases the *H*-cell is inhibited by GABA ; but this action is not similar to the characteristic transient hyperpolarization provoked by this drug in *D*-cells.

5-Hydroxytryptamine (5HT) excites the two types *H* and *D*, but its effect is more than ten times more intense on *D*-cells (Fig. 2*A*). 5HT is apparently present in the molluscan nervous system[5], and its participation in synaptic transmission has also been considered[6].

When the effect of drugs is studied by perfusing the whole ganglion, the true pattern of response of the membrane to the drug may be changed by the interference of interneurones, which are also affected by the drug. For example, 5HT, the primary action of which is excitatory, can indirectly inhibit an *H*-cell by exciting at the same time an inhibitory interneurone, thus producing inhibitory postsynaptic potentials in the impaled cell (Fig. 2*B*). This may be avoided by injecting the drug locally ; but the perfusion has the advantage of giving additional information about the action of the drug on the interneurone, the activity of which is reproduced in the synaptic pattern recorded in the cell by intracellular electrode. This method enabled us to establish that the inhibitory internuncial neurones, responsible for the inhibitory input on *H*-cells, are of the *D* type.

Conclusions from this work may be summarized as follows :

(1) Two types (*H* and *D*) of neurones with different and specific pharmacological properties are present in the central nervous system of *Aplysia*. These specificities are demonstrated by the response of the *H*- and *D*-cell membranes to acetylcholine, noradrenaline, adrenaline, 5HT and GABA.

(2) The type of pharmacological response is the same on the synaptic and extrasynaptic regions close to the cell body.

(3) The pharmacological pattern of the inhibitory interneurone corresponds to the *D*-type.

(4) These differences in pharmacological specificities of neurones with apparently no special localization in an elementary central nervous system show the difficulties presented by pharmacological studies of neuronal groups and the advantage of concentrating the pharmacological analytical research as much as possible on elementary structures.

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Apparent Absence of Amines in Cerebrospinal Fluid of Phenylketonurics

THE mechanism by which mental defect is produced in phenylketonuria is unknown. No correlation has been found in patients having this disorder between the degree of mental defect and blood-levels of phenylalanine or phenylpyruvate, or urinary excretion of phenylalanine, phenyllactate, phenylpyruvate or *o*-hydroxyphenylacetate[1]. The possible implication of biologically active amines has been examined in several papers. Mitoma *et al.*[2] administered *o*-tyrosine and *m*-tyrosine to experimental animals and observed conversion to the corresponding amines in brain, with attendant central nervous system stimulation and convulsions. Association of the mental defect in phenylketonuria with an excessive production of *o*-tyramine was suggested. It also seems possible that overproduction of phenylethylamine may be responsible for the disturbance in cerebral metabolism. Jepson *et al.*[3] have recently shown that administration of a monoamine oxidase inhibitor to phenylketonuric individuals results in an increase much greater than normal in the urinary excretion of phenylethylamine, but not of *o*-tyramine. The mental defect in phenylketonuria may also be due to an underproduction of amines vital to normal brain function. Pare *et al.*[4] have reported low serum serotonin-levels and decreased urinary excretion of 5-hydroxyindoleacetic acid in phenylketonuria. They suggest that one or more aromatic acid metabolites of phenylalanine inhibit 5-hydroxytryptophan decarboxylase, the enzyme responsible for the production of serotonin.

It was felt that the demonstration of the presence or absence of biologically active amines in cerebrospinal fluid might give clues to the mechanism of the mental defect in phenylketonuria. Because cerebrospinal fluid is formed in the ventricles and bathes the brain, it may be in a better position than other body fluids to reflect the biochemical changes occurring in the brain in disease. In the recently discovered disorder argininosuccinicaciduria[5], the concentration of argininosuccinic acid has been found to be greater in cerebrospinal fluid than in plasma, and may reflect a derangement in cerebral metabolism.

In the present work, 4 phenylketonuric children and 4 control children of similar ages having mongolism were given orally a potent monoamine oxidase inhibitor, β-phenyl*iso*propylhydrazine ('Catron', *JB*–516), in a dosage of 9–12 mgm. once daily for 12–14 days. Before and again after 12–14 days of monoamine oxidase blockade, 10 ml. of cerebrospinal fluid was obtained from each patient by lumbar puncture. Cerebrospinal fluid was stored at − 20° C. until used. None of the patients exhibited any consistent change in behaviour while receiving 'Catron', other than that expected incident to the discomfort of removing cerebrospinal fluid.

Specimens of cerebrospinal fluid were deproteinized by ultrafiltration at 5° C. for 24–36 hr. Amines were absorbed on the resin 'Amberlite *CG*-50', type 2 (H^+ form) according to the method of Awapara *et al.*[6]. Each filtrate of cerebrospinal fluid was shaken for 1 hr. with 1 gm. of resin on a mechanical shaker, the resin was washed with water in a column, and the bases were then eluted with 4 *N* acetic acid. The eluate was lyophilized, and the residue was dissolved in 0·5 ml. of 10 per cent aqueous 2-propanol.

The concentrates of cerebrospinal fluid were chromatographed one-dimensionally on Whatman No. 1 paper in butanol/acetic acid/water (12 : 3 : 5). The equivalent of 1 ml. of original cerebrospinal fluid was applied to each chromatogram. Individual chromatograms were prepared from each specimen for development with ninhydrin, diazotized *p*-nitroaniline, and *p*-dimethylaminocinnamaldehyde. As little as 0·05 μgm. of serotonin, 0·25 μgm. of *o*-tyramine and 0·25 μgm. of phenylethylamine could be detected readily. Graded amounts of these authentic amines were chromatographed simultaneously on the same sheet with each specimen of cerebrospinal fluid.

No amines were detected in 1 ml. of cerebrospinal fluid from each of the 4 phenylketonuric and 4 mongoloid patients, either before or during monoamine oxidase blockade. The unused portions of concentrates of cerebrospinal fluid obtained during monoamine oxidase blockade were then pooled, and most of the salts and basic amino-acids were removed by precipitation with ethanol and acetone according to the technique of Rodnight[7]. Again, no amines were detected on chromatograms prepared from 10 ml. of pooled phenylketonuric or pooled mongoloid cerebrospinal fluid.

The efficiency of the recovery procedure for amines in cerebrospinal fluid and the possibility that amines might be destroyed during deproteinization by monoamine oxidase naturally present in the cerebrospinal fluid were checked by adding authentic amines to cerebrospinal fluid. 2 μgm. of serotonin and 10 μgm. of phenylethylamine per ml. were added to cerebrospinal fluid obtained from phenylketonuric and mongoloid patients prior to monoamine oxidase blockade. The cerebrospinal fluid specimens were incubated for 4 hr. at 37° C. to promote possible enzymatic action, and then were deproteinized by ultrafiltration for 36 hr. at 5° C. The filtrates were processed and chromatographed as previously described. Recoveries of added serotonin and phenylethylamine were about 75 per cent.

The results show that cerebrospinal fluid of each phenylketonuric patient during monoamine oxidase blockade contained less than 0·05 μgm. of serotonin and less than 0·25 μgm. of phenylethylamine and *o*-tyramine per ml., and that pooled phenylketonuric cerebrospinal fluid contained less than 0·005 and 0·025 μgm. per ml. respectively of these amines.

The failure to detect amines in phenylketonuric and mongoloid cerebrospinal fluid during monoamine oxidase blockade may be due to insufficient sensitivity of the technique used. Published reports suggest that the concentration of amines in cerebrospinal fluid is very low, if indeed they are present at all. Sachs[8], using a biological assay method, found serotonin in cerebrospinal fluid of some patients with head injuries and brain tumours. It is possible, however, that this serotonin may have been derived from bleeding into the subarachnoid space. Weil-Malherbe and Liddell[9] determined epinephrine and norepinephrine fluorimetrically in cerebrospinal fluid, and found these amines present in average concentrations of 0·9 and 2·8 μgm./l. respectively. The possibility that, in the present experiment, amines originally present in the cerebrospinal fluid in low concentration were destroyed enzymatically has not been excluded, but this seems unlikely since the patients were given a potent monoamine oxidase inhibitor, and in view of the recovery of added authentic amines after incubation of cerebrospinal fluid.

It is likely that many intracellular biochemical alterations in the brain are not reflected in the cerebrospinal fluid. For example, γ-aminobutyric acid, present in relatively large amounts in the brain and the retina but not elsewhere in the body, is not normally found in cerebrospinal fluid. A failure to find amines in cerebrospinal fluid, even during monoamine oxidase blockade, may result simply from inability of these substances to penetrate from the brain or blood into the cerebrospinal fluid. Thus the results of the present work do not exclude the possibility that the mental defect in phenylketonuria may be related to an underproduction of serotonin, or an over-production of phenylethylamine or *o*-tyramine.

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Dynamics of Renal Proximal Tubular Secretion

A NUMBER of exogenous substances such as penicillin, 'Diodrast' and *p*-aminohippuric acid (PAH) are secreted in the proximal tubules. Nothing definitely is known about the mechanism of uptake of these substances in the tubular cells and further transport into the tubular lumen[1]. It is likely that enzymatic reactions are involved so that PAH reacts with an enzyme (carrier) in the cell membrane since the kidney cells seem to be impermeable to it. The concentration of PAH therefore approaches zero in the vicinity of the cell. However, it must traverse the wall of the capillary and the interstitial water before reaching the tubular cell. This probably takes place as diffusion along a concentration gradient. The concentration of PAH in the capillary plasma will therefore never be zero.

It is, however, not reasonable to explain in this way extraction of PAH (E) as low as 0·90 or lower at low plasma concentrations since similar extractions have been found for PAH and 'Diodrast' although these substances have very different diffusion coefficients[2]. When the flow of blood through the kidney is increased at unchanged arterial hæmatocrit, the extraction of PAH is considerably reduced (ref. 3 and unpublished work).

When blood with a low plasma concentration of PAH (c_0) reaches the glomeruli, fluid is filtered off, whereas the plasma concentration remains essentially unchanged. During the passage of the postglomerular capillaries the PAH concentration is reduced to $c_0(1 - E)$. The total amount removed and secreted into the tubular fluid is the sum of the amounts abstracted at all levels of the capillaries with decreasing concentration of plasma.

It is well known that the reaction velocity of enzymatic reactions, in this case the reaction which limits the transport-rate for PAH from interstitial fluid into tubular fluid, depends on the concentration of the substrate. The reaction velocity is low at low concentrations and increases to a maximum (V) which is uninfluenced by further increase in the concentration of PAH (c) delivered to the tubular cells. The relationship between reaction velocity and concentration of substrate is expressed in Michaelis's equation[4]:

$$v = Vc/(c + K_m) \qquad (1)$$

K_m is the Michaelis constant (the concentration of substrate at which the reaction proceeds at half the maximal velocity) and v is the amount of PAH abstracted from the capillaries per unit length and unit time. Since the concentration of PAH is decreasing during the passage of the capillary, it follows that:

$$v = -\mathrm{d}c/\mathrm{d}t \qquad (2)$$

where t is the time of passage through the capillary.

By combination of these two equations and integration for the time t between the limits for PAH concentration c_0 and $c_0(1 - E)$:

$$E + \ln(1/1 - E)K_m/c_0 = Vt/c_0 \qquad (3)$$

The rate of flow of plasma through the capillaries is inversely proportional to the passage time when the volume of the capillaries remains constant. The equation may therefore be rewritten:

$$E + K_m \ln(1/1 - E)/c_0 = K_0 f \qquad (4)$$

where K_0 is a constant and f is the flow-rate.

Maximal extraction values for PAH are obtained when its concentration is low (lower than K_m). The ratio K_m/c_0 is therefore larger than unity.

Fig. 1 shows that the extraction of PAH is reduced when the renal plasma flow-rate through the postglomerular capillaries is increased. The relationship between E and percentage increase of flow of plasma is in good accordance with experimental findings (unpublished). The basis for Fig. 1 is the assumption that the extraction is 0·8 at a renal plasma-flow of 200 ml./min. It is evident from the figure that the values for extraction of PAH at a given flow-rate are nearly identical whether the arterial plasma concentration for PAH is twice (filled circles) or ten times (open circles) smaller than K_m. This deduction is also in accordance with experimental findings.

This hypothesis explains why the extraction of PAH may be reduced without a similar reduction in Tm for PAH. At high plasma concentrations the amount removed will not vary with blood-flow. This can be shown by integrating the Michaelis equation with respect to capillary volume. The result is:

$$T - fK_m \ln(1 - T/fc_0) = K_1 \qquad (5)$$

where T is the total amount secreted per minute and K_1 is a constant. When the load of PAH ($fc_0 = L$) delivered to the tubular cells is large compared with the amount secreted, the second term in equation (5) vanishes, and T reaches its maximal value, usually called Tm. In titration curves for PAH the relation-

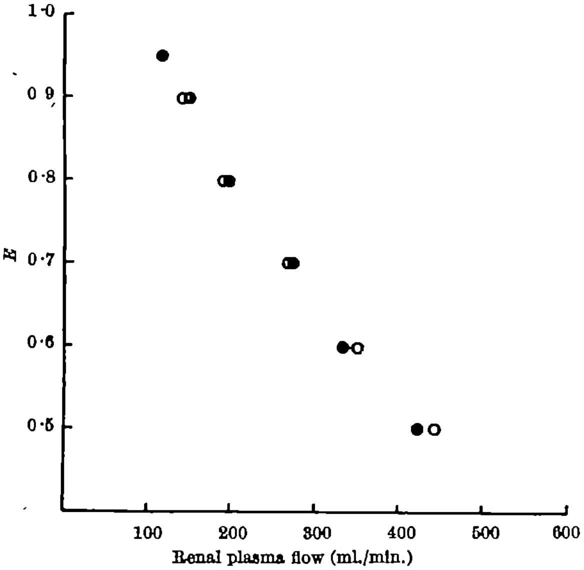

Fig. 1. Expected extraction of PAH at various rates of flow of plasma. ●, when $K_m/c_e = 2$; ○, when $K_m/c_e = 10$

ship between T/Tm and L/Tm is shown. The equation (5) may therefore be rewritten :

$$T/Tm - K_2 \log(1 - (T/T_m/L/T_m)) = 1 \qquad (6)$$

where K_2 is a constant when plasma flow is constant.

Fig. 2 shows the results for PAH-titration of a 53-year-old normal man[6], and the stippled curve is the titration curve for PAH as interpreted by Smith[6].

The other curve is plotted on the basis of equation (6), assuming $T/Tm = 0{\cdot}75$ and $L/Tm = 1$ as reference point. The shape of the curve obviously depends on this choice, and curves which fit the experimental data better may be found. According to equation (6) the tubular transport system is 90 per cent saturated when the load is slightly more than twice Tm, but 95 per cent saturation requires a load more than eight times larger than Tm. Tm is never reached, but only approached. As apparent from Fig. 2, Smith's and my estimates of Tm are different. The common assumption that there is no splay in the titration curve of an individual nephron obviously has to be reconsidered.

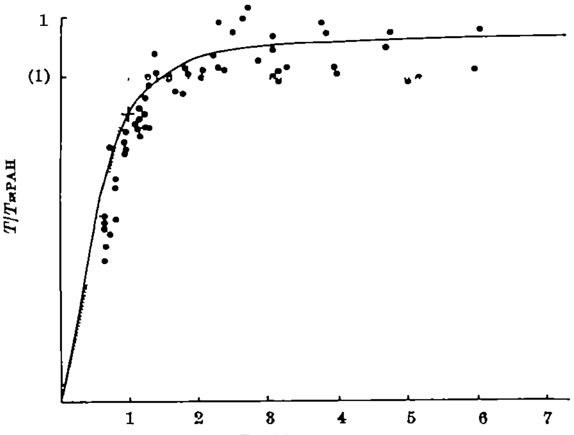

Fig. 2. PAH titration curve redrawn after H. Smith (ref. 6, 166). Solid line shows titration curve if Michaelis's equation is followed

A similar reasoning may be used for reabsorptive processes with a maximal reabsorptive capacity, but is more complicated since the decrease in concentration of the substrate during the passage of the proximal tubules is not only a function of the rate of reabsorption of the substrate but also a function of the considerable rate of water abstraction.

The model proposed suggests that the rate-limiting process for the transport of PAH follows Michaelis equation. This process is not necessarily localized in the cell membrane towards the tissue side, but evidently is a function of the substrate concentration in the interstitial fluid. The maximal rate of transport may therefore depend on the concentration of enzyme for the rate-limiting reaction. Competitive inhibition of transport processes may be interpreted along these lines. By means of titration curves for chemical analogues the constants V and Km in Michaelis's equation may be evaluated. The rate-limiting process may, however, not be the same for transport of different analogues.

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Minimum Rates of Flow in Therapy with Sulphonamides

Recent work on the kinetics of the acetylation of sulphisoxazole[1] and sulphamethylthiadiazole[2] in human beings indicate that excretion of acetylated drug is the terminal step in a two-step consecutive first-order process the first step of which is acetylation. This kinetic model was tested for ability to describe the metabolism and excretion of sulphathiazole taken by normal adult humans under a standard test procedure[1]. When 0 5-gm. doses were taken, excretion of acetylated drug was well described by the appropriate mathematical expression[1,2]. However, when one subject took a 1·0-gm. dose, it was not possible to describe the excretion of acetylated sulphathiazole by the same expression. The test was repeated with the same subject about two weeks later, and a water intake of about 500 ml./hr. maintained during the first 8 hr. of the test. This time excretion of the acetylated drug was well described by the expression. Using the rate constants obtained in the second test to calculate maximum excretion-rate of acetylated sulphathiazole by this subject from a 1·0-gm. dose, and comparison of this rate with the average urine flow-rate during the expected time of occurrence of the maximum during the first test, indicated that the solubility of acetylated sulphathiazole was exceeded at the average urine pH. It seemed reasonable to believe that crystalluria had occurred during the first test with a 1·0-gm. dose which would prevent description of excretion by the expression previously found to hold when a smaller dose was taken. There was no evidence of hæmaturia on casual gross examination of the urine specimens obtained in the first test with a 1·0-gm. dose.

The fact that the excretion of at least three acetylated sulphonamides[1-2] can be described in terms of several rate constants allows the following generalization to be made concerning minimum rate of flow of urine needed in therapy with these sulphonamides in order to prevent crystalluria.

Urine concentration of acetylated drug is given by:

$$Cu = \frac{dSae}{dt}\Big/\frac{dU}{dt} \qquad (1)$$

where Cu is the concentration in mgm./ml., $dSae/dt$ excretion-rate of acetylated drug in mgm./hr. and dU/dt average rate of flow of urine in ml./hr. It is usual in therapy to give an initial loading dose of drug, followed by maintenance doses at regularly spaced intervals. This procedure establishes blood-concentration and body-level at a nearly constant value. Excretion-rate of acetylated drug is given by:

$$\frac{dSae}{dt} = k_2Sa \qquad (2)$$

where k_2 is the first-order rate constant for excretion of acetylated drug in reciprocal hours and Sa is the amount of acetylated drug in the body at any time in mgm. The rate of change of acetylated drug in the body with time is:

$$\frac{dSa}{dt} = k_1g - k_2Sa \qquad (3)$$

where k_1 is the first-order rate constant for acetylation in reciprocal hours, and g is the constant amount in the body in mgm. For simplicity in mathematical treatment, it is assumed that the dose is instantaneously absorbed and equilibrium in body fluid is immediately obtained.

Equation 3 may be integrated and constant evaluated at zero time to obtain:

$$Sa = \frac{k_1g}{k_2}(1 - \exp[-k_2t]) \qquad (4)$$

Equation 4 reduces to the following at a time infinite in terms of the experiment:

$$Sa = \frac{k_1g}{k_2} \qquad (5)$$

It can be calculated on the basis of data available for the mean value of k_2 for sulphisoxazole[1] that equation 4 is within 95 per cent of the value given by equation 5 in 40 hr.; with sulphamethylthiadiazole[2] in 4 hr., and with sulphathiazole (unpublished work) in about 8 hr. Substituting equation 5 in equation 2 gives:

$$\frac{dSae}{dt} = k_1g \qquad (6)$$

Substituting equation 6 in equation 1 and rearranging gives equation 7:

$$\frac{dU}{dt} = \frac{k_1g}{Cu} \qquad (7)$$

If Cu in equation 7 is taken as the solubility of the acetylated sulphonamide at a given urine pH, then dU/dt becomes the minimum rate of flow of urine needed to prevent the occurrence of crystalluria.

Table 1. MINIMUM URINE-RATES OF FLOW (ML./HR.)

Urine pH	Sulphisoxazole	Sulphamethylthiadiazole	Sulphathiazole
5·0	190	90	420
5·5	60	60	400
6·0	20	40	370
6·5	10	20	280
7·0	5	10	170

Some calculations made with equation 7 are listed in Table 1. Solubility was calculated as a function of urine pH by the procedure of Krebs and Speakman[3] for sulphathiazole. Values for solubility of acetylated sulphisoxazole and acetylated sulphamethylthiadiazole at the several pH's were obtained from published values[4]. The mean value of k_1 used in each case was obtained from the work previously mentioned[1-2]. The value of g was taken as 1 gm. and, from equation 7, flow-rate is directly proportional to this quantity.

Calculation of minimum values of rate of flow assumes no supersaturation of urine can occur, and that precipitation is immediate when solubility is exceeded.

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HÆMATOLOGY

Reduced Fibrinolytic Activity of Atherosclerotic Sera caused by an Increase in Low-density Lipoproteins in Blood

RECENT work from several laboratories indicates abnormalities in bloods of atherosclerotic patients characterized by high cholesterol-level, elevation of cholesterol : phospholipid ratio and elevated low-density lipoproteins[1-3]. These abnormalities are reflected in a remarkably poor fibrinolytic activity of these bloods[4]. The work reported here was carried out in an attempt to find out the cause of low fibrinolytic activity of such bloods (collected from patients with advanced atherosclerosis), and to determine the nature of the material responsible for the inhibition. It is also hoped that such studies will enable us to find out the cause of frequent coronary deaths among older people after they have had fatty evening meals. The present work was based on analyses of blood specimens obtained from patients having a history of myocardial infarction, in the belief that these patients had developed atherosclerosis.

Blood samples collected from normal individuals (without known vascular or cardiac disease) between 30 and 40 years of age and from those with a history of coronary infarction were tested for fibrinolytic activity. The ability to dissolve a fibrin clot was used to measure the fibrinolytic activity of these blood sera. To each specimen an equal volume of highly purified fibrinogen[5] was added, mixed well and then thrombin was added to the mixture to produce clotting. The concentration of fibrinogen in the final solution was 0·2 per cent. Fibrinolytic activity was also measured in some cases after activation of the serum with streptokinase[6]. In general, the fibrinolytic activity of normal serum has always been found to be

Table 1. FIBRINOLYTIC ACTIVITY OF SERA

Normals			Atherosclerotics		
No.	Age	Percentage of clot lysis in 24 hr.	No.	Age	Percentage of clot lysis in 24 hr.
1	32	100	1	65	60
2	36	85	2	58	70
3	31	92	3	62	65
4	39	90	4	72	60
5	35	86	5	61	70
6	38	100	6	57	65
7	33	95	7	64	58
8	32	95	8	66	75
9	38	100	9	62	70
10	37	82	10	67	64

30–35 per cent higher than the corresponding values for sera collected from atherosclerotic patients (Table 1).

Chylomicra and low-density lipoproteins were removed from the sera with a Spinco model *L* preparative ultracentrifuge according to the method developed by Havel *et al.*[7].

The removal of chylomicra and low-density lipoproteins from the blood of atherosclerotic patients results in an increase in fibrinolytic activity (Table 2). The subsequent removal of α-lipoproteins from these blood samples does not alter their fibrinolytic activities. The removal of low-density lipoproteins thus renders these blood samples indistinguishable from normal bloods which have also been subjected to similar treatment.

Table 2. FIBRINOLYTIC ACTIVITY OF SERA BEFORE AND AFTER TREATMENT

Normals				Atherosclerotics			
No.	Age	Percentage of clot lysis in 16 hr.		No.	Age	Percentage of clot lysis in 16 hr.	
		before treatment	after treatment*			before treatment	after treatment*
1	32	70	100	1	65	45	95
2	36	60	95	2	58	50	100
3	31	65	90	3	62	48	100
4	39	75	100	4	72	42	95
5	35	60	86	5	61	46	95
6	38	80	100	6	57	45	88
7	33	75	100	7	64	40	90
8	32	75	100	8	66	38	90

* Serum was either treated with chloroform/methanol mixture or subjected to the method of Havel *et al.* in order to remove chylomicra and low-density lipoproteins.

Repeated treatments (5–6 extractions) of normal serum or serum obtained from atherosclerotic patients' blood with chloroform–methanol mixture (1 : 1 by volume), which removes chiefly the low-density lipoproteins, tends to raise the fibrinolytic activity of the atherosclerotic patients' blood to the value of the normal blood after similar treatments, although originally the fibrinolytic activities of those samples had been different (Table 2). These findings are in good agreement with those reported by Greig and Runde some years ago[4]. Similar treatments of the plasma with petroleum ether (b.p. 40–60° C.), which renders the plasma unclottable by Russell's viper venom and calcium[8,9], fail to alter the fibrinolytic activity of these blood samples. The poor fibrinolytic activity of the circulating blood can thus be attributed to the presence of low-density lipoproteins rather than any free phosphatides. Another point of interest is the fact that phospholipids extracted from the serum by the use of a chloroform–methanol mixture cannot inhibit fibrinolysis, at least not at the physiological concentration-level, since streptokinase-activated plasminogen (streptokinase and human serum) requires almost as much time to dissolve a fibrin clot in the presence of phospholipids as in the absence of this material.

Since 1940, it has been known that blood, after repeated treatments with chloroform, becomes fibrinolytic ; but it is not known what factor or factors (that can be removed by chloroform–methanol mixture) act as inhibitors of the fibrinolytic system of the circulating blood. One possible explanation might be that they form some type of inert complex with either plasmin or plasminogen, thereby preventing the former from acting on its substrate (fibrin clot) or the latter (plasminogen) from being spontaneously converted to plasmin. The fact that chloroform–methanol mixture can remove the factor or factors, acting as inhibitors in the circulating blood, implies their lipid nature. It is assumed that spontaneous conversion of plasminogen to plasmin is taking place all the time, although to a very limited extent ; the reason why there is no clot-lysis under these conditions is perhaps due to the presence of an inhibitor (antiplasmin), acting against plasmin. If this explanation is correct then chloroform–methanol mixture actually removes this inhibitor from the blood ; the fibrinolytic enzyme thus set free acts on fibrin clot (Fig. 1). The fact that serum-treated streptokinase exhibits much higher clot-dissolving activity than the chloroform–methanol-treated serum (Table 3), and also the fact that streptokinase cannot enhance fibrinolytic activity in bovine serum suggest that it must be acting in an entirely different way. The question is what it does then ?

Streptokinase, as is known, combines with a pro-activator present in blood to form an activator which then converts plasminogen to plasmin. The activator has all the properties of a proteolytic enzyme[10] ; and it is known that the conversion of plasminogen to plasmin can also be induced by trypsin or other mould enzymes[11]. Streptokinase has no proteolytic or esterase activity of its own ; most probably it removes some specific inhibitor (anti-activator) from the circulating blood thereby facilitating the conversion of plasminogen to plasmin by the activator thus set free. In this scheme the consideration of the hypothetical pro-activator is superfluous. The reason why bovine serum, after treatment with streptokinase, does not acquire any fibrinolytic activity might be due to the absence of the activator in such serum.

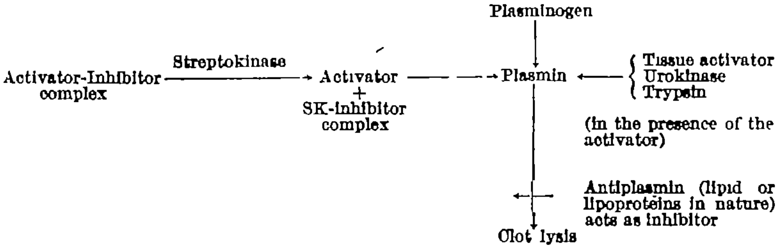
Plasminogen
Streptokinase
Activator-Inhibitor complex
Activator
+
SK-inhibitor complex
Plasmin
Tissue activator
Urokinase
Trypsin
(in the presence of the activator)
Antiplasmin (lipid or lipoproteins in nature) acts as inhibitor
Clot lysis

Fig. 1. Mechanism of action of streptokinase and chloroform/methanol mixture in the generation of fibrinolytic activity in blood.
Streptokinase removes the factor which inhibits the activator, whereas chloroform/methanol mixture removes the antiplasmin. Removal of low-density lipoproteins from serum is the removal of the antiplasmin

Table 3. Fibrinolytic Activity of Sera after Activation with Streptokinase

Normal group			Atherosclerotic group		
No.	Age	,Time for clot lysis	No	Age	Time for clot lysis
1	32	4m. 20s.	1	65	4m. 35s.
2	39	4 40	2	58	4 40
3	33	3 55	3	62	4 48
4	36	3 50	4	72	4 40
5	31	4 10	5	64	4 30
6	32	4 05	6	67	4 35

To each 0·25 ml. serum, 0 025 ml. streptokinase solution was added; this mixture was allowed to stand for 30 min. at 37° C. To this mixture 0 25 ml. purified fibrinogen solution was added and 0·025 ml. thrombin (10 units/ml.) was added to produce the clot. The time required for complete lysis at 37° C. was noted in each case.

Heavy fatty meals, particularly when eaten by atherosclerotic patients, will have, as would be expected, an immediate effect on fibrinolysis. An increase in the phospholipid content of the blood under these conditions has been reported[12] and their importance in clotting has been also demonstrated[9]. Under these conditions one might even expect a reduction in clotting time.

It thus appears that the primary cause of death among atherosclerotic patients after a heavy, fatty evening meal is the failure on the part of the circulating blood to cause lysis of small clots formed in small arteries. However, the positive effect of phospholipids on blood clotting must not be overlooked, since this effect is at least partly responsible for enhancing clot formation. The relative frequency of attacks among aged people of coronary thrombosis in the evening or after retiring may possibly be related to the above observations.

This work has been carried out in the Department of Mycology at the Institute of Laboratories, Forest Hill, Mass. I thank Mr. James B. Gray for providing me with facilities to carry out the work. I also thank Johnson and Johnson Research Foundation for financial support.

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BIOPHYSICS

Effects of Ultrasonic Radiation in *Escherichia coli B* using Fluorochrome Acridine Orange as a Vital Stain

Strugger[1], one of the pioneers who introduced acridine orange as a biological fluorochrome, observed that dead cells take up more of this dye, and under ultra-violet excitation fluoresce red, whereas living cells take up less dye and fluoresce green. This difference in the fluorescence is often used as a test for viability of cells and is referred to as 'Strugger effect'[2]. Loss of viability associated with red fluorescence has also been confirmed by Krebs and Gierlach[3] in their work involving radiobiological action on *Allium cepa* cells. Later work has shown that green acridine orange fluorescence in normal cells is to be associated with cell constituents rich in deoxyribonucleic acid[4]. It is also known from the observations of Beers *et al.*[5], Bradley and Wolf[6], and Ranadive and Korgaonkar[7], that deoxyribonucleic acid can form two complexes with acridine orange. One of these complexes is formed when the relative concentration of acridine orange to deoxyribonucleic acid is low, and exhibits strong green fluorescence and the other occurs when this ratio is high and shows orange or red fluorescence. Shift of fluorescence from green to orange is a critical step in this transition, whereas further change from orange to deep red is not sharp and occurs with further increase in concentration of acridine orange, without involving formation of new complexes. 'Strugger effect' may be explained in terms of these observations as a kind of damage to structures rich in deoxyribonucleic acid in the inactivated cells. This results in their high uptake of acridine orange to such an extent that the corresponding fluorescence is copper red. Few observations, however, are available to indicate the nature and extent of damage near the critical point when the shift of fluorescence from green to orange just occurs. A study of this nature in relation to viability was carried out by us on *E. coli* using ultrasonic waves for irradiation and the findings are reported here.

The ultrasonic generator used in this work consisted of a radio-frequency oscillator and a high-voltage power supply unit built in this laboratory by one of us (V. G. T.). The output of the oscillator was link-coupled to a barium titanate transducer having a resonant frequency 1·6 Mc./sec. The transducer was in the form of a tube 2 in. long and 1 in. inside diameter, into which the glass tubes containing bacterial suspensions were introduced. The transducer was kept immersed in transformer oil which acted as a coupling medium for ultrasonic energy from the transducer to the experimental material. The transducer was worked at its resonant frequency, which was continuously checked during the observations with a loosely coupled wave meter (General Radio type 556*A*). The power of the ultrasonic generator was controlled by using a 'Variac' in the primary circuit, and the monitoring was carried out continuously during irradiation with the help of a 2 5-amp. resonant frequency meter in series with the transducer and a vacuum tube voltmeter across it. A Rhode and Schwartz electronic multimeter type *URI BN* 1050 was used for the purpose. During all the exposures the current passing through the transducer was kept constant at 1 amp. and the voltage across it 10 9 V. under loaded conditions. The resonant frequency power fed to the transducer under these conditions worked out to be $\approx$ 0·25 watt/cm.^{2}. Even for the highest exposure employed the overall heating of the suspensions was within 2 deg. C.

Escherichia coli strain *B* obtained from the National Chemical Laboratory of India, Poona, was used. Inocula from fully grown agar slants were allowed to grow in nutrient broth (Difco), for 24 hr. at 37° C.

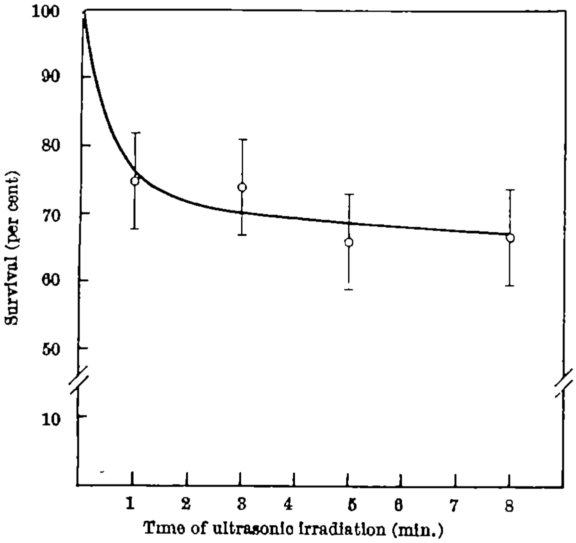

Fig. 1

The organisms were centrifuged, the pellet washed twice and finally suspended in 4·5 ml. buffered saline (pH 7·4). Aliquots of 0·5 ml. from such suspensions were diluted (1 : 10) and exposed to different doses of ultrasonic radiation maintaining suitable controls. The cells were assayed for viability by adopting the standard plating technique. Sterile conditions were maintained throughout the work and observations were repeated several times. The results of a typical set are shown in Fig. 1.

For the examination of fluorescence, measured quantities of control and treated bacterial suspensions were mixed with equal volumes of acridine orange solutions (conc. 0·05 per cent) in veronal buffer (pH 5·3). After an interval of 10 min., smear preparations of the stained material were made on glass slides (Gold-seal). The fluorescence of the vitally stained material was observed under a Reichert 'Lux U.V.' fluorescence microscope. A mercury arc lamp with a blue transmitting filter No. 8027 was used as a light source and blue cut-off filter No. 8006 was placed over the eyepiece.

In the untreated population, green fluorescence was observed, in almost all the cells. Preparations receiving even a few seconds exposure to ultrasonic radiation showed cells with orange fluorescence. Exact cell count was not possible under the limited magnification available with the fluorescence microscope. The occurrence of orange fluorescing cells increased with the dose, and with about 1 min. exposure almost the entire population turned orange. Further treatment did not show any noticeable change in this fluorescence within the dose-range covered in these experiments.

Some of the ultrasonically treated suspensions were inoculated into liquid media, allowed to grow, and observations on fluorescence were made as above on these. Orange fluorescence was noted, and cells with green fluorescence could scarcely be observed. Confirmatory evidence for the persistence of orange fluorescence in the subsequent generations was obtained from the observations on cells picked up from isolated colonies grown on nutrient agar plates, from the treated suspensions.

The salient points from these observations are: (1) Ultrasonic treatment at the intensity-level employed shows about 25 per cent loss in viability in *E. coli* after 1 min. of exposure and no appreciable further loss is noted even with prolonged treatment up to 8 min. (2) Such a treatment also causes a shift of fluorescence from green to orange on staining the cells with acridine orange. (3) An exposure of 1 min. is sufficient to bring about such a change in fluorescence in almost the entire population. There is no indication of a threshold dose even at the lowest dose employed. (4) The altered response to acridine orange staining persists in the subsequent generations observed.

From these findings it appears that if copper red fluorescence is to be associated with total inactivation of the cells as observed by Strugger and other workers, there is a wide gap in the cell damage between such a stage and the point close to the critical shift of green fluorescence to orange. More than 70 per cent of the cells showing orange fluorescence are found to retain their viability. Thus, the capacity for growth of the cell as a whole as well as the replication potential of its genetic material (which is considered to be chiefly involved in the fluorescence shift) is retained to a large extent. The cell at this stage seems to be physiologically closer to the normal cells than to the totally inactivated ones. Besides, there is an indication that it has acquired a degree of resistance to ultrasonic inactivation. The observed persistence of the altered response to acridine orange staining on sub-culturing is also interesting from its genetic implications and needs further investigation.

One of us (S. S. R.) expresses his thanks to the Council of Scientific and Industrial Research (India) for the award of a research fellowship, during the tenure of which this work was carried out.

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RADIOBIOLOGY

Retention of Zinc-65 in the Bones of Rats

The distribution and retention of zinc-65 in the organs and tissues of both normal and tumour-bearing animals has been studied extensively[1-4]. Most of these observations, however, have extended only over short periods following administration of zinc-65 and little attention has been paid to the retention of the isotope in bone. Ballou[5] and Gilbert and Taylor[6], in work extending over periods of up to 300 days, have shown that there is a prolonged retention of zinc-65 in bone.

In the present investigation the retention of zinc-65 in the bones of rats has been observed over

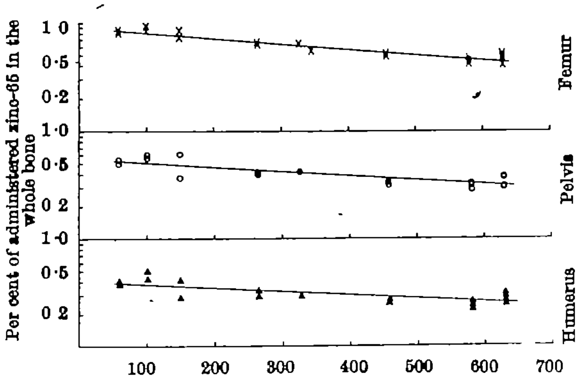

Fig. 1. Retention of zinc-65 in the femur, pelvis and humerus of 'August' rats

a period of 630 days following intravenous injection of the isotope.

The animals used were male rats, seven weeks old, and of the highly inbred, brown-hooded, August strain. Each animal received 2 μc. zinc-65 administered intravenously as a 'zinc-65 serum protein complex'. The preparation of this complex and other experimental details have already been described[6]. The animals were killed in pairs at varying times after injection and the femur, humerus, pelvis and ribs were assayed for zinc-65 and total zinc content.

The retention of zinc-65 in the femur, pelvis and humerus over the period from 58 to 630 days after injection is shown in Fig. 1. In each case it will be seen that, over this period, the retention can be described by a single exponential function. The equation for each bone, together with the appropriate half-times and the standard error of the rate constants, are given in Table 1.

Table 1

Bone	Equation	Half-time (days)	Regression coefficient ± *S.E.*
Femur	$0·91\,e^{-0·00116t}$	598	0·00116 ± 0·00012
Pelvis	$0·56\,e^{-0·00092t}$	752	0·00092 ± 0·00015
Humerus	$0·40\,e^{-0·00074t}$	941	0·00074 ± 0·00017
Mean	—	738	0·00094 ± 0·00024

The specific activities of zinc-65 in the various bones are shown in Table 2. It will be seen that, 7 days after injection, the zinc-65 has been distributed approximately uniformly throughout the zinc contained in the skeleton. The total zinc content of all the bones which were examined continued to rise throughout the period of observation; the values shown in Table 3 indicate that the zinc content of the femur, pelvis and humerus, expressed as μgm. zinc/gm. of bone, do not differ significantly from each other at any age. The zinc content of the ribs does not increase as much as that of the other bones.

Table 3. VARIATION OF ZINC CONTENT OF BONES WITH AGE

Age (days)	Femur	Humerus	Pelvis	Ribs	Mean (excluding ribs)
56	77 ± 7	83 ± 5	70 ± 6	85 ± 6	77 ± 3
103	104 ± 2	99 ± 5	101 ± 6	83 ± 4	102 ± 2
149	102 ± 5	116 ± 6	103 ± 18	71 ± 0	107 ± 6
198	105 ± 8	117 ± 13	102 ± 2	112 ± 7	110 ± 5
313	133 ± 8	130 ± 15	146 ± 3	105 ± 8	136 ± 5
679	191 ± 5	198 ± 11	211 ± 8	149 ± 12	200 ± 4

Zinc content of bone, μgm. zinc/gm. wet bone ± standard error All values are means of results from 2 animals except values for 679 days. These represent the mean for 4 animals.

The half-times for the retention of zinc-65 in the three bones are probably not significantly different from each other, although it appears that retention in the humerus is more prolonged than in the other two bones. The half-time reported here for retention in the femur is somewhat greater than the value which may be inferred from the results reported by Ballou for the retention of zinc-65 in the femur of Sprague–Dawley rats.

The mean half-time for the retention of zinc-65 reported here is much shorter than the corresponding half-time of 1,610 days for the retention of calcium-45 in the bones of rats of the same age and strain (Taylor, D. M., unpublished observations).

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Assessment of Dietary Strontium-90 through Urine Assay

SINCE the manner in which the body handles ingested strontium is quite well established[1–3], the chief problem of determining the relative strontium-90 hazard from food concentration data is that of defining the overall average diet with precision. Thus the usual problems concomitant with obtaining dietary intake for nutritional or other purposes apply. In addition to this there is the rather severe problem, generally of minor importance with respect to nutrients, of selecting for assay representative aliquots

Table 2. SPECIFIC ACTIVITY IN PERCENTAGE OF INJECTED ACTIVITY/MGM. ZINC

Time (days)	7		58		100		149		264		630			
Animal No.	1	2	3	4	5	6	7	8	9	10	18	19	20	21
Femur	42 39	37 30	13·1 12·7	12·2 11·2	11 4 11·4	11·8 11·8	7·4 —	9·1 9·1	4 9 4·8	5 6 6·2	2·3 2·5	1·9 1·8	2·4 2 3	2·3 2 3
Humerus	31 33	32 34	11·3 13·2	11 3 10·9	13·9 13·7	9 5	6·5 6 5	9·2 11·3	6·1 6 2	5·7 5·6	3 1 2·3	3 5 3 1	3 0 2·6	2·7 —
Pelvis	40	34	12·1	11·5	13·5	10·0	7·1	10·3	3·7	5 0	2·1 2·1	1·7 1 6	1·6 —	1·8 2·0
Ribs	27	27	15·1	10·0	11·9	13·5	8·3	6·1	4 0	4 9	0·7	1·1	0 5	1·1

of individual food items. Whereas differences in the vitamins or mineral content by a factor of two are rare for a given food, differences in strontium-90 content by a factor of ten or more are not uncommon[4].

The variations due to latitude[5] and rainfall[6] have long been recognized. In addition, the pattern of rainfall during the growing season is also critical, particularly with respect to cereals[7]. Thus wheat grown on fields only a few miles apart may differ markedly if one got a local shower two weeks before harvest and the other did not.

In view of these problems, it was proposed to use urinary concentration as an independent index of strontium-90 ingestion. By virtue of interchange with soft tissues and other labile strontium-90 depots, such a sample would represent some averaging of the ingestions over a period of days, although the most recent meal would play the most dominant part. Such a sample would also circumvent the problem of getting diet information which may be biased due to the presence of the observer. One can bring additional food to a few meals or think of extra items for an interrogator with much more facility than he can change his excretion-rate.

A study of dietary strontium-90 in particular military groups, together with its concentration in corresponding urine, has been conducted in three countries in co-operation with the Interdepartmental Committee on Nutrition for National Defense of the National Institutes of Health and Department of Defense. The Committee regularly conducts dietary surveys in connexion with nutrition studies in various countries around the world. Representative composite diets are obtained for nutrient assay and aliquots of these have also been used to determine dietary strontium-90. In a number of the local investigations of military units large samples (approximately 12–15 litres) of urine were also obtained, these being random collections representing a large number of men in each case.

Comparative data on diet and corresponding urine for various locations in Peru, Ecuador and Vietnam are given in Table 1. It is noted that the food/urine concentration ratio in terms of μμc. strontium-90/gm. calcium is approximately 2 on the average in each of the three countries investigated. The relation is about the same on the rice diet of Vietnam, which is relatively high in strontium-90 as in Peru and Ecuador. It is concluded from these results that for monitoring purposes strontium-90 concentration in urine gives a reasonable index of the dietary level.

Table 1. COMPARISON OF STRONTIUM-90 CONCENTRATION IN FOOD AND URINE. μμc. STRONTIUM-90/GM. CALCIUM

	Food	Urine	Ratio
Peru			
Arequipa	2·72	1·31	2·1
Lima	2·49	1·72	1·4
Piura	1·75	0·93	1·9
Tumbes	2·53	1·21	2·1
			Ave. 1·9 ±0·2
Ecuador			
Cuenca	1·02	0·67	1·5
Guayaquil	1·54	0·54	2·9
Loja	3·95	1·41	2·8
Machala	3·71	1·50	2·5
Quito	0·84	0·45	1·9
Salinas	3·00	1·35	2·2
			Ave. 2·3 ±0·4
Vietnam			
Bien Hoa	3·5	4·0	0·9
Quang Trung	6·9	3·7	1·9
Duc My	13·8	3·9	3·5
Danang	17·0	8·5	2·0
			Ave. 2·1 ±0·7

The question of which of the two indices is the more reliable under these conditions of field operations cannot be answered on the basis of the present findings ; but there is an indication that the urine values may be the better of the two. Whereas the mean deviation of the individual ratios from the average is 12 and 18 per cent in Peru and Ecuador, it is 33 per cent in the case of Vietnam. This may reflect two special difficulties encountered with the Vietnam diet. In Vietnam the diet is composed of more than 80 per cent rice and, as noted, grains in particular fluctuate markedly in their strontium-90 content. Thus an individual food composite taken on a given day would be less representative of the overall picture than the same sample taken in Western culture or South America. Secondly, small fish, shrimp, etc., which commonly make up a portion of the Vietnam diet, are typically incorporated into the 'stew' and may be eaten, bones and all. Strontium-90/calcium ratio in fish is generally very low compared with other foods, and thus this fluctuating parameter would also reduce the validity of short-term composite diet data with respect to the strontium-90 component.

It is concluded that : (1) composite food and urine data on the population groups reveal that the food/urine concentration ratio of strontium-90 in terms of μμc. strontium/gm. calcium is about two ; (2) for monitoring purposes, the urine strontium-90 concentration is a satisfactory index of ingestion. Urine data may be the more reliable under some conditions of field observation.

This work was supported by the U.S. Atomic Energy Commission under contract *AT* (30–1)–1656. The food and urine samples were collected by survey teams of the Interdepartmental Committee on Nutrition for National Defense. Team members of particular note in this phase of the survey included Jerome Uram and Ogden Johnson (Peru), Gerald Combs and Alvin Wolfe (Ecuador) and C. Frank Consolazio and Albert N. Booth (Vietnam).

The strontium-90 analyses were conducted by Rieta Slakter Hirshman and Karin Bartelsen of the Lamont staff.

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HISTOCHEMISTRY

Detection by Fluorescence of Oxytetracycline bound to Bone

Milch, Rall and Tobie[1,2] observed the property of antibiotics of the tetracycline series of becoming localized specifically in bony tissue formed during the administration of these drugs, and persisting in the zone of localization until the structural remodelling has caused the absorption of the bony areas which have bound the tetracyclines. This observation seemed likely to offer a practical and useful method of vital staining, especially for the detection of the turnover of bone.

In fact, the tetracyclines, which are fluorescent in ultra-violet light[3], preserve this property even when bound to bony tissue, and therefore it is possible to detect them by fluorescence microscopy[1,2]. Obviously, if the time pattern of the administration of the drugs is known, it is possible to determine directly the age of the fluorescent bony structures and indirectly the value of the bone-forming activity and its topographical distribution.

As the tetracycline bound to bone is dissolved with bone salts, histological methods which imply decalcification must be avoided. Consequently, the observations of Milch *et al.* were carried out on undecalcified sections prepared by a freezing rotatory microtome; the limited size of sections obtained by this method inevitably is a disadvantage.

I found that fixation in formaldehyde, in alcohol, or in acetone modifies, but does not destroy, the fluorescence of oxytetracycline bound to bone. Thus I was able to use the method of Sognnaes *et al.*[4], embedding the specimens in methyl methacrylate and preparing sections ground with emery paper. In this way the sections obtained are thin enough for the perfect detectability of fluorescence in the microscope.

All this is strictly necessary when the specimens to be examined consist not only of bone but also of soft tissues. The details of this method, which permits the study of sections of unlimited size, together with the technique used in fluorescence microscopy, have been described elsewhere[5].

The results obtained with experimental material and from biopsy and necropsy specimens reveal that the method of vital staining of bone with tetracyclines has a significant advantage over the common method of labelling bone with isotopes. In fact, the optical resolution in fluorescence microscopy is much higher than that allowed by the granular form of autoradiographic emulsions.

In sections prepared by embedding in methyl methacrylate and grinding, the fluorescence is distributed in different patterns according to the structure of the bony tissue. In actively growing bones mainly of a parallel-fibred, finely bundled structure, one can observe yellow fluorescent areas alternating with other areas without yellow fluorescence.

In adult animal and human bone, which mainly consists of lamellar bone, one can observe at times large and homogeneously yellow fluorescent rings or bands, composed of various 'lamellæ', and at other times, thin yellow fluorescent rings or lines, arranged singly or serially, composed of only one 'lamella'.

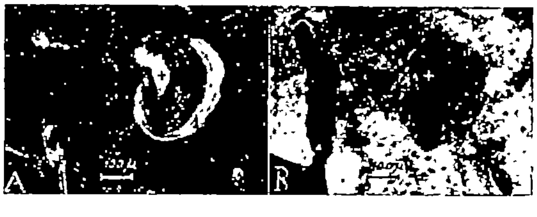

Fig. 1. Undecalcified section, 50μ thick, from the radius of a three-year-old dog, which received three successive administrations of oxytetracycline. (*A*) shows the fluorescence in white; (*B*) is the microradiograph of the same field. The first administration of oxytetracycline is represented in (*A*) by three fluorescent parallel rings (1), the second by two fluorescent rings (2), the third by the fluorescence of the central osteoid edge of the Haversian system (+)

As a rule, different values of mineralization correspond microradiographically to the differences in age as revealed by the fluorescent areas (see Fig. 1).

In adult human lamellar bone, the persistence of tetracycline fluorescence localized in bony tissue has been observed more than seven months after administration, with some variations in intensity and colour of the emitted fluorescent light. This persistence is determined by the quickness of the remodelling process and, perhaps, by differences in mineralization patterns of bony tissue.

These results will be discussed more fully elsewhere.

This method, with its specificity and its advantages as compared with the radioisotope-labelling method, can be appropriately applied to the study of any physiological and pathological bone problems which require a precise knowledge of the age of the structures. It would indeed be very useful to have a complete understanding of the chemical nature of the binding of tetracyclines to bone, as this would allow a better explanation of the mineralization of the bone.

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Histochemistry of Human Sublingual Glands

BIOLOGICAL work on salivary glands has revealed that sialic acid is the only acidic component in the bovine submaxillary gland and probably in the sublingual gland of the rat[1-3]. These findings resulted in the elaboration of a method for the removal of sialic acid in tissue sections known to contain abundant amounts of this sialo-mucin[4,5]. Such a method consisted of exposing the sections to acid hydrolysis at different *p*H's and temperatures.

It was then decided to evaluate human sublingual glands histochemically using alcian blue, safranin *O*, and azure *A* for acidic polysaccharides and periodic acid–Schiff for glycoproteins. The glandular mucous acini always showed metachromasia with azure *A* and safranin *O* and a great affinity for alcian blue and periodic acid–Schiff.

Sections of tissue were then incubated in Walpole buffer solutions at *p*H 2·5 at 55° C. and 80° C. for 2–16 hr. It was found that only incubations at 80° C. for 16 hr. resulted in a slight loss of alcian blue and azure *A* staining, and considerable diminution in safranin *O*-positive material.

In an attempt to classify the nature of the substance or substances that presumably were not affected by mild acid hydrolysis, sections were exposed to the action of testicular hyaluronidase for varying periods of time of 1–3 hr. This procedure did not reverse metachromasia or the affinity for alcian blue, thus indicating that the acinar material contained sulphated polysaccharides other than chondroitin sulphate *A* and/or *C*, which are readily hydrolysed by hyaluronidase. In order to determine whether chondroitin sulphate *B* or a closely related substance was present in the acini, tissue sections were then incubated in 1 *N* hydrochloric acid at 80° C. for 10, 30 and 60 min. This treatment caused only a partial decrease in staining.

Because chondroitin sulphate *B* may be removed by acid hydrolysis, these findings would seem to suggest that human sublingual glands contain a heparin-like substance alone or in addition to a sialo-mucin.

Incubations of tissue sections in papain and pepsin for periods of time up to 1 hr. did not affect the periodic acid–Schiff, alcian blue and metachromasia, while prolonged incubations (3 hr.) diminished the staining reactivity of the acini. These findings are opposite to those observed in bovine submaxillary and rat sublingual glands, in which even short incubations with either proteolytic enzyme completely removed all the staining materials. The observations relative to human sublingual glands would indicate that significant species differences exist in the composition of salivary mucins. These results are also of interest in the light of the results reported by Pusztai and Morgan[6] on their work on the effects of papain and ficin on blood-group-specific mucopolysaccharides. These authors demonstrated that dialysis following exposure to proteolytic enzymes failed to reveal demonstrable amounts of sugars and oligosaccharides.

These findings, together with the observation by Tasiro[7] that human sublingual gland is one of the richest sources of blood-group substances, suggest the possibility that some of the polysaccharides in human sublingual mucin are closely related to blood-group substances.

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PATHOLOGY

Induction of Melanotic Tumours in the White ('Partial Albino') Syrian Hamster

DELLA PORTA *et al.*[1] first reported the induction of melanotic tumours in the skin of Syrian golden (agouti) hamsters after topical applications of 7,12-dimethylbenz(*a*)anthracene (DMBA). This finding was afterwards confirmed by these and other investigators[2–5]. Ghadially and Barker[5] have postulated that the melanotic lesions arise from dermally situated perifollicular networks of melanocytes intimately associated with certain hair follicles. They suggest that the proximity of dermal melanocytes to sebaceous glands of hair follicles renders them especially vulnerable to the action of carcinogens topically applied to the skin. Recently, Illman and Ghadially[6] have reported the induction of melanotic tumours in the general body skin of white ('partial albino') Syrian hamsters during repeated topical applications (22 weekly treatments) of 2 per cent DMBA in acetone. It is noteworthy that white hamsters characteristically possess a white hair coat, and grossly detectable pigmentation is restricted to the skin of the ears, perineum and genitals; a darkening of eye colour may occur with advancing age[7,8]. Whiteness of hair coat is a genetically determined trait inherited as a simple Mendelian recessive[7,8]. The purpose of this communication is to report observations made in our laboratory on the occurrence of melanotic lesions in white hamsters treated with DMBA.

In our experiments, initiated without knowledge of those of Illman and Ghadially[6], 10 male and 10 female random-bred white hamsters, 4 months old at the start, were treated once weekly for 3 consecutive weeks with a 1 per cent solution of DMBA (Eastman) in mineral oil, essentially the same procedure as that of Shubik *et al.*[3]. Before the first treatment, the hair was shaved from the backs of all animals by means of electric clippers. Each treatment consisted of the delivery by pipette of 4 evenly spaced drops of the carcinogenic solution to this region. It was estimated that each drop of solution represented a delivered volume of 20–30 µl.; that is, it contained 200–300 µgm. DMBA.

Raised nodules were observed in the treated areas 15 weeks from the start[3]. Undoubtedly, they were present prior to this time, for some lesions were already 3–5 mm. in greatest diameter. The vast majority did not exceed a diameter of 2 mm. and in many cases appeared flat to gross inspection. By the eighteenth week, more nodules were evident, and many of the older ones had increased in size. The nodules ranged in colour from pink-white through brown to an intense black. Certain lesions recorded as pink on first observation were noted as grey-black 3 weeks later. Both light and dark nodules were present in the same animal, and there was no clear correlation between nodule size and gross pigmentation. Of 20 hamsters, 19 survived to the fifteenth week after treatment. Of these, 18 had at least one nodule at that time. The remaining hamster, a male, showed evidence of raised nodules by the eighteenth week after treatment.

Shubik *et al.*[3] have designated pigmented nodules exceeding 2 mm. as melanotic tumours. At the time of writing (20 weeks), 8 surviving females have a total of 34 spots 2 mm. or more in greatest diameter on the dorsum, including the tail; the corresponding value for 8 surviving males is 44. Although occasional lesions now exceed 6 mm. in diameter, most lesions are as yet smaller than 2 mm. The total population of dorsal spots on surviving hamsters is now 99 for the females and 112 for the males. In addition, 11 pigmented lesions have been noted in or near the margins of the eyelids. These lesions vary from deep red to black in colour, and one is now approximately 7 mm. in diameter. Such lesions have not been reported previously for the white or pigmented hamster. It is possible that the spread of the carcinogen through wiping would account for the occurrence of pigmented lesions outside the areas directly treated with DMBA. No association of the costo-vertebral spots with pigmented lesions has as yet been noted.

Microscopically, the dorsal lesions appear quite similar to those described by Illman and Ghadially[6] for the white hamster, and by Della Porta *et al.*[1] and Ghadially and Barker[5] for the golden (agouti) hamster. A variable content of melanin granules would appear to be directly correlated with gross observations of the colour of the lesions. As previously reported[4], the pigment granules react positively with silver staining (Fontana) and are bleached by potassium permanganate. The lesions are highly vascularized, and are composed of cells mainly polyhedral or spindle-shaped. They are clearly dermal in location, although they intimately invest hair follicles[5,6]. The association of the melanotic tumours with hair follicles is especially evident in serial sections of skin where care is taken to obtain longitudinal sections of hair follicles. In some instances, the impression is gained that the lesions merge with follicular structures. The proximity of melanotic lesions to hair follicles provides further evidence that the pilosebaceous apparatus is an important channel for the entry of topically applied carcinogens into skin[8,9].

Nevertheless, the origin of melanotic lesions in the white hamster is as yet uncertain, for perifollicular networks of melanocytes have not been identified in this variety by Illman and Ghadially[6] or by us. It has been suggested[6] that melanotic tumours in white hamsters are derived from a system of amelanotic perifollicular melanocytes. Indirect evidence supporting this view is our preliminary observation that repeated topical applications of croton oil result in an increased number of perifollicular melanocyte nests in the golden hamster. Thus it would appear that dermal melanocytes may be present in an amelanotic state, at least in this variety of hamster. The possibility that melanotic lesions are derived from hair follicles cannot as yet be entirely excluded. This explanation has been given by Szabó[10] for the origin of melanotic spots in pigmented mice treated with DMBA and croton oil. It is hoped that the present experiments dealing with the effect of repeated topical applications of croton oil on white hamsters will shed some light on this problem.

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Toxicity and Anti-leukæmic Effectiveness, in Mice, of 4-Amino-4-deoxy-N^{10}-methyl-5,6,7,8-tetrahydropteroylglutamic Acid (Tetrahydroamethopterin)

IT has recently been reported[1] that the 5,6,7,8-tetrahydro derivatives of the folic acid antagonists aminopterin, N^{10}-formylaminopterin and amethopterin have a greater inhibitory action on the growth of *Streptococcus faecalis R* and *Pediococcus cerevisiae* than the unreduced analogues. It was therefore of interest to determine whether the greater inhibitory action found in the bacterial system is also reflected in a mammalian system, by an increase in host toxicity and/or anti-leukæmic activity.

Amethopterin and its tetrahydro derivative were chosen for a comparative study. The former is used regularly in our Laboratory[2] as a standard drug for the evaluation of new anti-leukæmic agents, and its toxicity to normal mice is well established. Tetrahydroamethopterin was prepared according to the method of Kisliuk[1,3], and for injection was dissolved in a vehicle of 0·6 per cent potassium ascorbate in 2 per cent aqueous sodium bicarbonate. The solution was stored frozen in rubber-sealed vials under hydrogen in the dark. Under these conditions, tetrahydroamethopterin appears to be stable for several days. Amethopterin was also dissolved in the ascorbate–bicarbonate mixture.

Hybrid male *CDBA* mice [(*BALB/cAn* × *DBA/2J*)F_1], 12–13 wk. old and weighing 24–28 gm. at the beginning of the experiment, were used in this work. Normal mice, mice bearing advanced leukæmia *L*1210 (amethopterin-sensitive) and mice bearing advanced leukæmia *L*1210–*M*46*R* (amethopterin-resistant)[4] were divided into groups and given daily subcutaneous injections of the drugs at 0·01 ml./gm. body-weight. Vehicle and untreated controls were included in each experiment. Records were kept of the weights of the animals and, in the tumour-bearing animals, of the local tumour size.

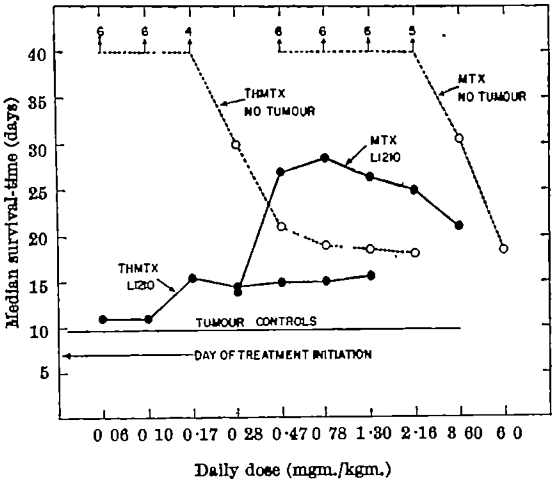

Fig. 1. Toxicity and anti-leukæmic effect of amethopterin (MTX) and tetrahydroamethopterin (THMTX). ○, normal mice, 6 per group; ●, tumour mice, 10 per group. The tumour mice were inoculated subcutaneously in the right inguinal region with about 2×10^6 cells of leukæmia *L*1210 on day 0. Daily treatment was initiated on day 7 after inoculation and continued until the end of the experiment. Treatment of the normal mice was begun on the same day as that of the leukæmic mice. The numerals above the vertical arrows refer to the number of mice in each group which survived until day 40, at which time the experiment was terminated

Table 1. EFFECT OF CITROVORUM FACTOR (CF) ON THE TOXICITY OF AMETHOPTERIN (MTX) AND TETRAHYDROAMETHOPTERIN (THMTX) IN NORMAL MICE

	Drug administered alone			Drug administered concomitantly with 20 mgm./kgm./day CF		
Drug	Dose (mgm./kgm.)	Survivors* day 14	Median survival time (days)	Dose (mgm./kgm.)	Survivors* day 14	Median survival time (days)
MTX				160	0	9·5
				80	4†	> 14
	40	0	5	40	6	> 14
	20	0	5	20	6	> 14
	10	0	8	10	6	> 14
	5	1†	12	5	6	> 14
	2·5	6	> 14	2·5	6	> 14
	1·25	6	> 14			
THMTX				80	0	9·5
				40	5†	> 14
				20	6†	> 14
	10	0	11·5	10	6	> 14
	5	0	9·0	5	6	> 14
	2·5	0	10·0	2·5	6	> 14
	1·25	0	10·5	1·25	6	> 14
	0·625	1†	13·5	0·625	6	> 14
	0·31	6†	> 14	0·31	6	> 14
	0·16	6	> 14			

* Six mice per group; each group received daily treatment from day 0 to day 13.

† Mice receiving these dosage-levels had lost considerable body weight by day 14. Other studies of the toxicity of MTX and THMTX in normal mice (Fig. 1) suggests that these animals would have succumbed to continued treatment.

Fig. 1 shows the results obtained for normal mice and mice bearing advanced leukæmia *L*1210. The results obtained with advanced leukæmia *L*1210–*M*46*R* are not included as neither drug caused an increase in survival time over the untreated or vehicle controls, at any of the doses indicated. This would seem to indicate a complete cross-resistance for the tumour to the tetrahydroamethopterin. The toxicity of tetrahydroamethopterin on a daily treatment schedule, in normal mice, was about ten times that of amethopterin. The toxicity of both drugs was manifested by a 25–30 per cent body weight loss prior to death, and the marked melæna observed would indicate severe gastrointestinal mucosa damage (more marked in the case of tetrahydroamethopterin).

With advanced leukæmia *L*1210, amethopterin gave a typical bell-shaped curve when survival time was plotted against logarithm of dose[4]. In contrast, tetrahydroamethopterin gave a flat curve over a similar dose range (0·17–1·30 mgm./kgm./day), and the survival-times in this region were considerably less than for the optimal dose of amethopterin (0·78 mgm./kgm./day). In a second experiment with the tetrahydro derivative a similar flat curve was obtained, and the median survival-time of the mice did not decrease until a dose of 52 mgm./kgm./day was used.

Although both drugs brought about a reduction of the local tumour size during the course of treatment, an effective dose of tetrahydroamethopterin, for example, 0·17 mgm./kgm./day, was attended by considerable loss of weight of the host. This is in contrast to amethopterin, where an effective dose, for example 0·78 mgm./kgm./day, caused no loss of weight over the same time span. Thus, it appears that toxicity to the host is a severely limiting factor in the treatment of advanced leukæmia *L*1210 with tetrahydroamethopterin.

Since previous work has shown[5] that the toxicity of folic acid antagonists, in mice, can be nullified by the concomitant administration of citrovorum factor, but not by injecting folic acid at the same time, it was of interest to determine whether the same relationships existed between tetrahydroamethopterin and these metabolites. To this end, a series of normal *CDBA* mice was given daily doses of amethopterin or tetrahydroamethopterin ranging from non-toxic to toxic levels. A parallel series received 20 mgm./kgm./day of citrovorum factor (calcium leucovorin) with the drug. The results are shown in Table 1. Citrovorum factor gave complete protection from frankly toxic doses of both drugs. However, in a second experiment in which 20 mgm./kgm./day of folic acid was administered concomitantly over the same dose ranges of the drugs, no protection from toxic doses of either drug was observed. The above observations suggest that amethopterin and tetrahydroamethopterin are acting by similar mechanisms in so far as their toxicity to normal mice is concerned.

It is also of interest to note that the diminished antileukæmic effectiveness of tetrahydroamethopterin, relative to amethopterin, is paralleled by a diminished ability of the drug to inhibit purine biosynthesis

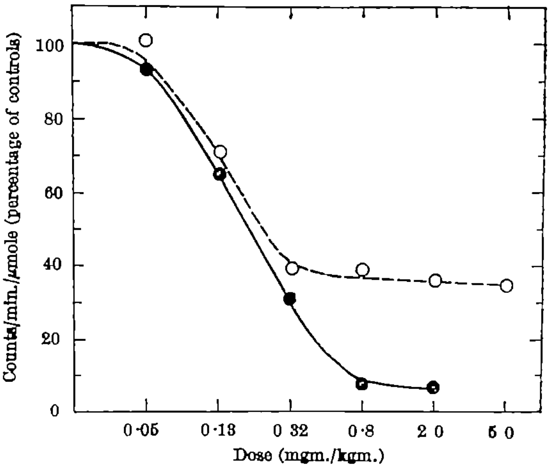

Fig. 2. Effect of amethopterin (●) and tetrahydroamethopterin (○) upon the incorporation *in vivo* of carbon-14 labelled formate into the acid-soluble adenine of mouse spleen infiltrated with leukæmia *L*1210. The experiment was carried out on day 8 after leukæmic inoculation. The mice were given a subcutaneous injection of the drug, then 20 min. later an intraperitoneal injection of 200 μc./kgm. of carbon-14 labelled sodium formate, and were killed after another 20 min. Acid-soluble adenine was isolated from the pooled spleens of three mice, and its specific activity determined (ref. 6). Untreated controls received 2 per cent sodium bicarbonate solution. Results are expressed as

$$100 \times \frac{\text{counts/min./}\mu\text{mole (treated)}}{\text{counts/min./}\mu\text{mole (controls)}}$$

de novo. Previous investigations in our Laboratory have shown[4] that the inhibition of carbon-14 labelled formate incorporation *in vivo* into the acid-soluble adenine of leukæmia-infiltrated mouse spleen can be related quantitatively to the dose of amethopterin administered to the animals. Fig. 2 shows the dose-response curves obtained with amethopterin and tetrahydroamethopterin in this system. It can be seen that, at low doses, both drugs produced similar partial inhibition. However, beyond a level of about 0·32 mgm./kgm. of tetrahydroamethopterin, no increased inhibition was observed. This is in contrast to the marked inhibition produced by higher doses of amethopterin.

The present work has shown that tetrahydroamethopterin is more toxic to normal mice than is amethopterin at equivalent dose-levels. In mice bearing advanced leukæmia *L*1210, tetrahydroamethopterin also appeared to cause somewhat more extensive regression of the local tumour than amethopterin. However, the increased toxicity of tetrahydroamethopterin was proportionately greater than the anti-tumour effect, so that there was a net loss of anti-leukæmic effectiveness. Thus, the toxicity of tetrahydroamethopterin relative to amethopterin for host and tumour cells is, in fact, similar to that reported in the bacterial studies[1]. The biochemical basis for this greater toxicity of tetrahydroamethopterin is not known. One possibility is that it is a more effective antagonist of the reduced, coenzymically active forms of folic acid than amethopterin.

We are doing work on the biological and biochemical activity of the reduced folic acid antagonists.

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Ovine Fœtal Resorption caused by *Toxoplasma gondii* Infection

Wastage in ovine reproduction due to infection of the pregnant ewe by *Toxoplasma* has been described in New Zealand[1,2], Australia[3,4] and the United Kingdom[5]. Experimental infection has on several occasions[1,6] confirmed its role as a cause of perinatal mortality. Following experimental infection, ovine toxoplasmosis has now been observed to cause resorption of the fœtus in addition to perinatal mortality.

Experimental infection of ewes, which were 101–122 days pregnant, was effected by intraperitoneal inoculation with undiluted peritoneal exudate from infected mice. Nine ewes were thus infected and kept under surveillance in a small enclosed pen until parturition. Following inoculation, three ewes showed evidence of a sanguineous discharge at the vulva and were therefore killed.

In one ewe, autopsied sixteen days after inoculation, twin fœtuses, which were partially resorbed, were still distinguishable and the placentomes remained intact, although the intercotyledonary component of the fœtal membranes had disappeared. The placentomes showed lesions of focal necrosis similar to those typically seen in cotyledons from infected fœtal membranes[4].

The two other ewes were not autopsied until 30 and 40 days after inoculation, at which stage no fœtus or fœtal membranes remained obvious, however, there was considerable enlargement of the caruncles, these being concave and approximately 1 in. in diameter; a quantity of sanguineopurulent material was also present within the uterus. In view of the fact that the ewes had been under constant observation since inoculation, these findings were taken as presumptive evidence of resorption.

There is no published information concerning total prenatal mortality-rates in flocks in which toxoplasmosis is known to occur naturally; however, results from a Dorset Horn flock in south-eastern Queensland in which perinatal mortality due to toxoplasmosis has been diagnosed, indicate a large discrepancy between the number of ewes apparently conceiving and those eventually lambing. This discrepancy is only partly accounted for by abortions. It is suggested, therefore, that in some circumstances, fœtal resorption may be more important than perinatal mortality as a manifestation of ovine toxoplasmosis.

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BIOLOGY

Genetic Control of Oxytetracycline Biosynthesis

More than ten years ago Bonner[1] made an unsuccessful attempt to use inactive variants and biochemical mutants of *Penicillium chrysogenum* for studying the mechanism of penicillin biosynthesis. Since that time the mechanism of the biosynthesis of the antibiotic has apparently not been investigated in this way.

In 1956 we published a paper[2] on the biosynthesis of oxytetracycline by methionine- and niacine-requiring mutants of *Act. rimosus*.

It was shown that both mutants require for the antibiotic production much greater amounts of these substances than is needed for their growth. Repeatedly isolated mutants with methionine and niacin deficiencies had the same properties. These results convinced us of the necessity of investigating the genetical control of the mechanism of the antibiotic biosynthesis, particularly the biosynthesis of oxytetracycline. In this connexion an attempt was made to obtain balanced inactive mutants of the oxytetracycline-producing organism.

At first, under the action of mutagenic factors, namely, ultra-violet light, X-rays or ethyleneimine, inactive variants were obtained, which practically lost the ability to produce the antibiotic ('zero' variants).

Two groups were observed among a great number of zero variants. One group took the form of colonies with very light to almost cream substrate mycelium and white aerial mycelium ('white' group). The other group formed colonies with dark brown substrate and greyish aerial mycelium, which excreted dark brown to almost black pigment into the culture fluid ('black' group).

When two mutants of the 'white' group were grown together on a liquid complex or synthetic medium, they produced no oxytetracycline, whereas when one mutant of the 'white' group was grown together with a mutant of the 'black' group, the activity markedly increased (Table 1). The plating of the mixed culture in Petri dishes showed that only the starting strains when grown together participated in fermentation. The second examination of the mutants with respect to their activity in a pure culture gave no positive results.

The nature of the antibiotic substance, synthesized on mixed submerged cultivation of two inactive variants of *Act. rimosus*, was studied by means of paper chromatography according to the method described by Selzer and Wright[3]. The antibiotic studied proved to be oxytetracycline.

The results of the determination of the antibiotic produced with the help of both the biological and the colorimetric methods coincide.

The studies on cross-transfers of the mycelium throws some light on the mechanism of interaction between the inactive mutants during the oxytetracycline synthesis. The mycelium of the 'black' mutant *T*572, separated from the culture fluid and washed with sterile water, was transferred into a 48-hr. culture fluid of the 'white' mutants *T*12 and *T*999, separated from the mycelium by centrifugation and filtered through a bacterial filter. In all cases of transferring the mycelium of a 'black' mutant into the culture fluid of a 'white' mutant the biosynthesis of oxytetracycline was observed, while in cases of transferring the mycelium of a 'white' mutant into the culture fluid of a 'black' mutant the synthesis was not observed. The behaviour of the 'black' mutant mycelium transferred into the culture fluid of the active starting strain of *Act. rimosus* was the same (Table 2).

Table 1

Strain	Corn steep liquor medium		Synthetic medium	
	Activity (γ/ml.)	Mycelium weight (mgm. %)	Activity (γ/ml.)	Mycelium weight (mgm. %)
Active starting strain	3,046	1,041	1,900	930
*T*1 (white)	0·55	1,095	0·50	1,042
*T*2 ,,	0·20	485	0·20	285
*T*11 ,,	0·41	1,160	0·55	852
*T*12 ,,	0·90	1,360	0·54	1,020
*T*16 ,,	0·80	1,012	0·70	940
*T*956 ,,	0·29	932	0·22	886
*T*999 ,,	0·50	1,042	0·48	787
*T*572 (black)	20·0	872	0·87	752
*T*1 + *T*572	1,030	1,075	790	890
*T*2 + *T*572	790	1,038	600	898
*T*11 + *T*572	830	1,002	490	897
*T*12 + *T*572	1,000	1,083	780	874
*T*16 + *T*572	600	990	600	854
*T*956 + *T*572	720	1,088	670	798
*T*999 + *T*572	760	930	600	870
*T*2 + *T*956	5·0	860	—	—
*T*2 + *T*999	4·0	956	—	—
*T*12 + *T*956	1·0	924	—	—
*T*12 + *T*999	3·0	926	—	—
*T*16 + *T*956	4·0	656	—	—
*T*16 + *T*999	4·0	905	—	—

Table 2

Combination of the mycelium and the culture fluid	Mycelium weight mgm. %		Activity (γ/ml.)			
	Initial	After 36 hr.	Initial	After 12 hr.	After 36 hr.	After 60 hr.
Culture fluid of *T*12 + mycelium of *T*572	1,290	1,384	0·1	106	480	520
Culture fluid of *T*999 + mycelium of *T*572	1,379	1,468	0·2	130	390	420
Culture fluid of *T*572 + mycelium of *T*12	1,520	1,504	0·8	1·0	0·9	1·0
Culture fluid of *T*572 + mycelium of *T*999	1,580	1,600	0·2	0·4	1·0	0·9
Culture fluid of the active starting strain + mycelium of *T*12	1,424	1,431	230	220	260	210
Culture fluid of the active starting strain + mycelium of *T*572	1,214	1,260	270	400	580	640

On the basis of this fact it is possible to assume that 'white' mutants produce some unknown substance (x-substance), which provides the antibiotic synthesis by the mycelium of the 'black' mutant *T*572. It is interesting to note that x-substance is present in the culture fluid of the active starting strain of *Act. rimosus*.

The task of further investigations is to elucidate the nature of this substance.

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Modifying Factors of Amino-acid Antagonisms in a Mutant of *Neurospora crassa*

IN certain amino-acid-requiring mutants of *Neurospora crassa*, any one of a number of naturally occurring amino-acids can inhibit competitively the utilization of the required amino-acid for growth[1,2]. In the course of determining the inhibition indexes of the inhibitory amino-acids, it was noted that variations in certain experimental conditions would cause significant differences in the observed indexes. The mutant strain *E*-5212 (ref. 3), which responds to either phenylalanine or phenylpyruvate, was used in a study of the various modifying factors. Growth assays were performed in the usual manner using Fries minimal medium[4]. The inhibition index is defined as the ratio of inhibitor to required amino-acid which produces complete inhibition of growth for approximately three days at 27° C.

A mutant strain of *Neurospora crassa* has been described which requires thiamine or thiazole for

Table 1. EFFECT OF pH AND TEMPERATURE ON INHIBITION INDEXES

L-Phenylalanine (µmole)	Inhibition index (L-leucine)	Temperature (° C)				L-Phenylalanine (µmole)	Inhibition index (L-valine)	pH		
		20	25	30	35			5·2	5·6	6·0
0	0	0	0	11	36	0	0	0	0	0
0·5	0	24*	39	65	68	0·1	0	44*	47	46
0·5	10	7	24	38	52	0·1	5	42	46	44
0·5	15	0	0	22	48	0·1	10	40	43	38
0·5	20	0	0	0	43	0·1	20	40	44	0
0·5	40	0	0	0	32	0·1	40	30	0	0
0·5	100	0	0	0	25	0·1	80	0	0	0
0·5	200	0	0	0	22					

* Figures indicate mgm. dry weight.

optimal growth at temperatures below 30° C. but which grows on minimal medium like the wild-type strain at 35–39° C.[5]. This mutant is inhibited by threonine, and the inhibition is most strongly expressed at 35° C. and at pH values below 5·8 (ref. 6). Table 1 shows that changes in pH and temperature had a much different effect on the amino-acid inhibitions described here than on the threonine inhibition previously reported[5,6]. The inhibition index of L-leucine was 15 at 20° C., but at 35° C. complete inhibition of growth could not be achieved with an inhibition index of 200, the highest index tested. The inhibition index of L-valine increased four-fold over the pH range 6·0–5·2. A mixture of purines and pyrimidines also modified the inhibition index of L-valine. When the components of the mixture were tested individually, the modifying effect was exhibited by two of the purines, guanine and xanthine, as shown in Table 2.

Table 2. MODIFICATION OF INHIBITION INDEX OF L-VALINE BY GUANINE AND XANTHINE

Inhibition index (L-valine)	5 µmoles						
	None	Adenine	Guanine	Uracil	Thymine	Xanthine	Hypoxanthine
0	40*	40	37	44	41	37	45
2·5	46	42	37	41	44	39	46
5	43	40	34	40	39	37	48
10	43	41	16	40	41	33	49
20	39	20	0	36	38	0	43
40	0	0	0	0	0	0	0

* Figures indicate mgm. dry weight. All flasks contained 0·1 µmole L-phenylalanine.

Mathieson and Catcheside[7] have reported that the growth of a histidine-requiring mutant of *Neurospora crassa* is inhibited by certain amino-acids in combination with lysine or arginine. If, however, the inhibitors were added after two days growth, they had no effect. *E*-5212 exhibited a somewhat similar response to post-inoculation additions of the inhibitory amino-acid, as shown in Table 3. All growth flasks were inoculated at zero time and contained 0·5 µmole of the required amino-acid, L-phenylalanine. The inhibitory amino-acid, L-leucine, was added at the indicated times. It can be seen that by only 8 hr. incubation the mutant was less sensitive to the inhibitory amino-acid. The mutant became increasingly resistant to the inhibitor with time, so that by 18 hr. an inhibition index of 400 was insufficient to inhibit growth completely.

The same effect can be demonstrated by measuring oxygen uptake by the usual manometric methods in a Warburg apparatus. The reaction vessels each contained an equal conidial inoculum and 0·5 µmole of L-phenylalanine in Fries minimal medium. Fifty µmoles of L-leucine was tipped in from the side-arm at the times shown in Fig. 1. By 12 hr., the inhibition was of such a nature that the rate of uptake quickly returned to that shown by the cells with no inhibitor present.

Table 3. EFFECT OF ADDING INHIBITORY AMINO-ACID POST-INOCULATION

Inhibition index (L-leucine)	Post-inoculation time (hr.)							
	0	4	8	12	18	25	36	48
0	41*	40	42	41	43	42	41 (9)†	40 (19)†
10	25	34	35	37	34	38	39	43
20	0	0	14	27	29	34	38	40
100	0	0	0	11	20	26	37	37
400	0	0	0	0	14	23	36	33

* Figures indicate mgm. dry weight after 72-hr growth. All flasks contained 0·5 µmole L-phenylalanine.
† Growth (mgm.) at designated time.

The primary result of the single genetic block in *E*-5212 is the requirement of phenylalanine or phenylpyruvate for growth. On further study, however, it has been shown that growth of the mutant on phenylalanine is subject to inhibition by any one of 14 amino-acids[3]. We have demonstrated here that the amino-acid inhibition, and consequently growth of the mutant, is in addition sensitive to a number of environmental variables. It has not yet been determined if the amino-acid inhibitions observed in *E*-5212 are the result of competition for entry into the cell or of competition for an intracellular site. It is suggested, nevertheless, that the site must be of such a nature that its affinity for phenylalanine and the inhibitory amino-acid is subject to the modifying factors discussed here.

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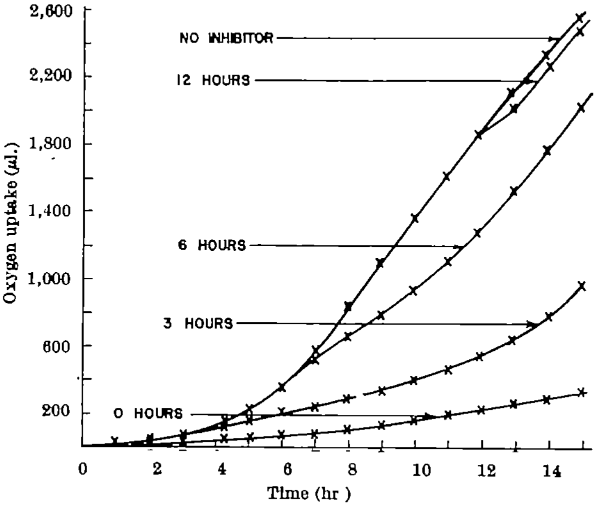

Fig 1. Effect of inhibitory amino-acid on oxygen uptake. Vessels contained 0·5 µmole L-phenylalanine. 50 µmoles of L-leucine was tipped in at the times indicated

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Response of Japanese Quail to Restricted Lighting

Recent publications on light response of the domestic fowl have directed renewed attention to the fact that a hen's reactions to experimental light stimulation depend, in part, on the conditions prevailing before a given light treatment[1–4]. Short-day lighting of immature pullets may lead to increased rate of egg production immediately following maturation, depending both on the length of pretreatment and light intensities used. Similarly, it has been shown that laying hens held in complete darkness will resume high rates of lay afterwards, even under light intensities considered insufficient when given without a preceding dark period[5]. The work in ref. 5 indicated that short but frequent doses of intense light may over-stimulate hens so that subsequent treatment with continuous light results in reduced rates of lay. A similar experiment with Japanese quail (*Coturnix coturnix japonica*) is reported here, in an effort to establish its usefulness as a pilot animal for the fowl.

Approximately 300 *Coturnix* were reared to 5 weeks of age. They belonged to three closed populations, all derived from the same original stock, but each in their first generation of selection for egg number. Genetically these populations may, therefore, be considered alike. The chicks were brooded in a quail battery and were given weak all-night light from a red 7-W. bulb for the first three weeks. They were then placed in chick batteries under natural daylight. At 5 weeks the birds were sexed and the females moved to single cages with 6 in. × 8 in. floor space ; each population was in a separate location. Two of these were windowless chambers equipped with a light-trap at the entrance. The third room, reserved for the control treatment, was located in an open chicken house. Experimental light treatments were first applied at 6 weeks of age when a few hens had already begun to lay eggs.

Group *A*, the control, was given 14 hr. of light every day, at an intensity of more than 1 foot-candle. Group *B* was given no light, except for such light as required when marking egg cards above the cages. This was done by means of a weak pencil flashlight, shielded to avoid direct exposure of the birds to light. Group *C* was given 1 min. of intense light of approximately 2 ft.-c. every hour. These conditions were maintained until the birds were 12 weeks old. At that time the light regimes of groups *B* and *C* were reversed, giving light at the rate of 1 min./hr. to hens preconditioned in darkness and vice versa. No change was made for Group *A*. At 16 weeks the birds were weighed and placed on normal light.

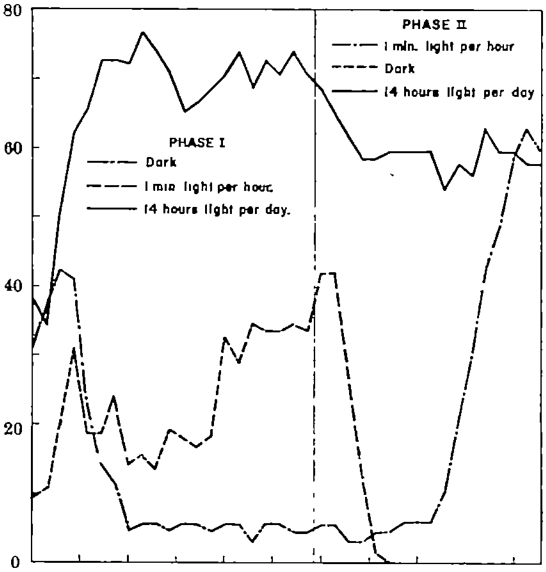

Fig. 1. Dependence of rate of lag on light regime

The response of the hens' rate of egg production to changes in light pattern is shown in Fig. 1. At 6 weeks of age the populations had started to lay at somewhat different rates. The onset of experimental lighting forced most of the hens of Group *B* treated in the dark out of production and prevented those not laying from beginning to do so. Within 2 weeks the rate of production in this group dropped to less than 10 per cent. Similarly, there was some reduction in rate of lay for Group *C* birds under intermittent lighting ; after about 2 weeks this population began a consistent rise in lay up to about 40 per cent by the sixth week of treatment.

After the switch of light regimes the two experimental groups changed their egg-laying pattern radically. Group *B*, previously in darkness, showed a rapid rise in egg production 2 weeks after the change to intermittent lighting. Within 4 weeks these hens had reached or exceeded the rate of lay of control birds. In contrast, the group held in darkness following treatment with intense intermittent light stopped egg production completely within a week of change in conditions, and stayed out of lay beyond 16 weeks of age.

In order to reach a general interpretation of these results, one may consider each type of experimental lighting first in its role as direct stimulus and, secondly, in its function in preconditioning the hens for subsequent lay. One may further consider the period of maturation before 6 weeks of age as preconditioning the hens with 14 hr. of light per day.

Viewed in this manner, the results suggest that intermittent lighting stimulates egg production of hens less than 14 hr. of light per day, but more than complete darkness does. It also seems clear that intermittent lighting following dark treatment is much more effective in its stimulatory role than after pretreatment with 14 hr. of light per day. Conversely it would appear that dark treatment reduced egg production more drastically after hens were preconditioned with intermittent lighting than was the case following the 14 hr. of light a day of the maturation period.

One may conclude that dark treatment of *Coturnix* while almost completely preventing egg production during its application may serve to enhance afterwards the response of hens to light. In contrast, the intermittent light regime permits only sub-optimal productivity unless preceded by a dark period or perhaps other treatment; it has adverse effects on the subsequent ability of hens to lay in darkness, when compared to normal 14 hr. of light per day. These conclusions are in general agreement with results obtained for chickens[1].

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Presence of Blood Antigens in Fish Mucus and its Possible Parasitological Significance

WE have recently shown in this laboratory that each species of fish appears to secrete its own specific mucus[1]. The differences between mucus from various species can be detected by chromatographic methods. With closely related species, like *Sebastes marinus* L. and *S. mentella* Travin, several types of chromatographic technique may be required in order to detect any difference between the two forms of mucus[2]. At present we know very little about the nature of this species specificity of the mucus. Some of the chromatographically separable components fluoresce on exposure to ultra-violet radiation and some are ninhydrin-positive.

Fish serum proteins are, of course, species specific, and therefore we have used some specific anti-fish sera to test for the presence of serum protein components in body and gill mucus. The recently described cellulose acetate membrane immunodiffusion method of Consden and Kohn[3], which we have already shown can be used to distinguish between the closely related *Sebastes* species mentioned[2], was used in this work.

Anti-bass (*Labrax morone* L.) serum *R* 923 was produced by injecting bass serum into a 2-kgm. male albino rabbit by a course of subcutaneous injections according to the following procedure: A preliminary injection of 0·5 ml. bass serum was followed after a week by a second similar one with another three days later and a fourth two days later still. The rabbit was bled out under nembutal, a week after the last injection. The antiserum so produced gave a homologous titre of 1 in 12,000 and a photronreflectometer sigma tau value of 152. Oudin[4] and Ouchterlony[5] immuno-diffusion studies showed that it contained at least six distinct antibodies to the homologous serum. Two of these antibodies reacted with components in *Sebastes marinus* serum and one reacted with cod (*Gadus callarias* L.) serum. No heterologous reaction with coalfish (*Gadus virens* L.) serum was detected. Further studies of bass blood are being reported elsewhere[6].

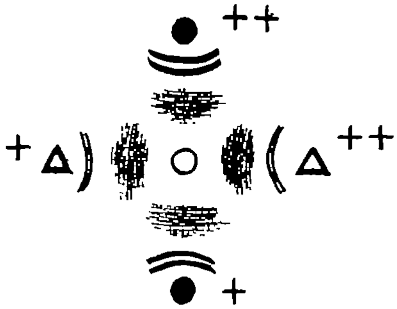

Fig. 1. Diagram of Consden–Kohn reactions between antibass serum *R* 923 (○) and bass mucus (●). Upper and lower reactions are with body mucus and left and right reactions are with gill mucus (△). Plus signs indicate approximate quantities applied. The inner broad bands are nigrosine-positive, the outer lines of precipitation are both ponceau *S*- and nigrosine-positive

When the anti-bass serum *R* 923 was treated with bass mucus using the Consden–Kohn cellulose acetate membrane technique it was found that three antigen antibody (*AnAb*) reactions occurred (Fig. 1). The two outer *AnAb* reactions were detectable by ponceau *S* staining which produced fine clearcut lines of reaction. The more diffuse innermost zone of precipitation was not detectable with ponceau *S*, but required the use of nigrosine staining. It was also found, as can be seen in Fig. 1, that the gill and body mucus reacted somewhat differently. The reactions were stronger with body mucus than with gill mucus. This is perhaps surprising since gill mucus might be more likely to be contaminated with blood than body mucus. It should be pointed out that in the samples studied no contaminating blood was detected. Furthermore, the ponceau *S*-positive *AnAb* reactions were well separated from each other whereas with gill mucus the two lines of precipitate, although discrete, were very close to each other. This would indicate that the relative concentrations of the two antigens involved differ in gill and body mucus.

Anticod serum *R* 712 available from previous work on intragenic relationships in the genus *Gadus*[7] was used to study the body mucus of *Gadus callarias*. Only a single *AnAb* reaction faintly ponceau *S*-positive was found. Subsequent staining with nigrosine gave no further reactions, but the ponceau *S*-positive band stained more strongly.

These results show that some of the serum protein antigens of fishes are also secreted in mucus. These species-specific antigens may be responsible, in part at least, for the ability of certain fish parasites to detect their specific hosts. Wright[8] has pointed out that such parasites must detect their hosts by chemotactic methods, and the presence of species-specific serum proteins in the body mucus suggests a possible basis for this.

Further work is in progress.

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Occurrence of a Species of *Halammohydra* on the Isles of Scilly

DURING the period March 24–28, 1959, collections of the interstitial fauna on several shores of the Isles of Scilly were made. On the very sheltered muddy sand shores of the islands of Tresco and St. Martin's, a small number of specimens of the cœlenterate, *Halammohydra* Remane, were recorded, in the surface 5 cm. of sand. The samples were treated with formalin immediately after sampling and this rendered exact identification impossible.

Attempts by one of us (L. A. H.) to collect live specimens at Easter, April 9–23, 1960, were unsuccessful. It may be, therefore, that the earlier presence of the animals was accidental, the true habitat being in the sub-littoral. Alternatively, it may be that these animals exhibit a seasonal cycle and were much less frequent at the time of the 1960 sampling.

The specimens exhibit certain differences from any of the known species, the number of tentacles varying from 9 to 15. Swedmark[1] points out that regional differences occur in the various species.

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Slugs at Low Temperatures

DAMAGE by slugs in gardens and farms is usually worst in late autumn and early spring. There are three reasons for this. First, some species are most numerous in winter[1]. Secondly, alternative foods are scarce, and crop plants such as germinating wheat or the young shoots from delphiniums are sometimes almost the only food available. Thirdly, slugs are active at temperatures which would put most cold-blooded animals into chill-coma.

Experiments have shown that different species of slugs have different chill-coma temperatures. *Agrolimax reticulatus*, which is incidentally a most important pest on farms, is particularly well adapted to life at low temperatures. It is not completely immobilized even at 0° C., and at 0·8° C. moves and feeds apparently normally. *Arion hortensis*, an important garden pest, seldom moves spontaneously or feeds below 5° C. *Milax budapestensis* is intermediate in its temperature relations to the other two species, for it is active at 5° C., but is inactive at 0·8° C.

Some years ago I showed that many insects[2], and also amphibians[3], change their chill-coma temperature by several degrees centigrade within 24 hr. when kept at different temperatures. Similar results for fish, though with a longer period of acclimatization, are well known. I fully expected that slugs would behave similarly.

However, *Agrolimax reticulatus* and *Arion hortensis* do not seem to show any low-temperature acclimatization at all. The chill-coma temperature seems to be quite unaffected if these slugs are kept for from 1 to 10 days at (*a*) 5° C. at (*b*) a temperature fluctuating between 0° C. and 10° C., and at (*c*) a constant temperature of 20° C. The explanation for this difference from insects, fish and amphibians has not been found, but slugs seem well adapted to life at low temperatures and do not readily lose this adaptation. The observations of Dainton[4] that dormant slugs become active, in the laboratory, when the temperature falls, is obviously relevant, but the behaviour of these animals under natural conditions in the field needs much further study.

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Trapped Primary Product of Photosynthesis in Green Plants

BY use of a suitable method of flash photometry five types of absorption changes (types 0, 1, 2*a*, 2*b*, 3) have been observed during photosynthesis in green plant cells. Measurements under various physical and chemical conditions lead to the following assumptions on the related reactions[1].

The absorption change type 1 is caused by a photochemical reaction on chlorophyll Chl a_1. Probably a metastable state of chlorophyll Chl $a_1^{\bullet}$ ($\tau \approx 5 \times 10^{-5}$ sec.) is formed, which mediates an electron transfer between an electron-donator D and an electron-acceptor A :

$$D(\text{Chl } a_1)A \xrightarrow{h\nu} D(\text{Chl } a_1^{\bullet})A \longrightarrow D^{+}(\text{Chl } a_1)A^{-} \quad (1)$$

The absorption changes type 2 were first observed by Duysens[2] using a static measuring method with excitation of photosynthesis by steady light; equal absorption changes result from the application of flash photometry[1]. These absorption changes are characterized by two negative maxima (~ 420 mμ, 475 mμ) and one positive maximum at 515 mμ. The course of the decrease of absorption as a function of wave-length in the neighbourhood of 420 mμ is comparable with the course of an oxidation of cytochrome[2].

Kinetic experiments carried out by flash photometry led to the conclusion that the absorption changes type 2 ($\tau = 10^{-2}$ sec.) are involved in the primary reactions of photosynthesis[1]. Further we succeeded in splitting up the absorption changes type 2 into two reactions, designated as type 2*a* and type 2*b*, by extraction with cold petroleum ether[3,4]. After extraction the absorption changes at 475 mμ and 515 mμ (type 2*b*) disappear, while a negative absorption change with a shift to ~ 425 mμ still can be seen. Due to the absence of type 2*b*, it is possible to detect a small positive absorption change near ~ 530 mμ which is connected with that at ~ 425 mμ (type 2*a*).

The results of our first measurements of absorption changes at low temperatures were reported at a meeting to discuss fast reactions in solution at Hahnenklee[5] (1959). The reaction type 1 (production of Chl $a^{\bullet}$) occurs at −150° C. in just the same way as at + 20° C. Therefore an electron transfer according to reaction (1) should also be possible at −150° C. The following observations on type 2*a* give evidence of this fact.

On illumination of spinach chromatophores at −150° C. the absorption changes; type 2*b* (475 mμ and 515 mμ) does not occur. The reaction type 2*a*, however, continues to occur at −150° C. On exposure to light, a decrease in absorption occurs at ~ 425 mμ (Fig. 1). Coupled with this decrease at ~ 425 mμ is a small increase at ~ 530 mμ, coincident with the observations made after extraction with petroleum

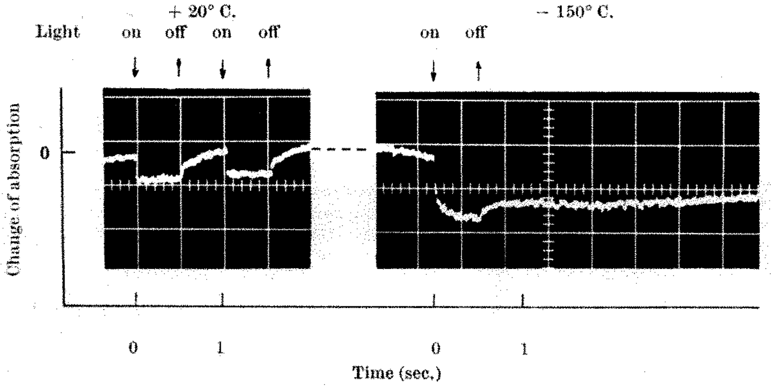

Fig. 1. Changes of absorption at 425 mμ caused by red illumination of chromatophores of spinach

ether. After switching off the light, the absorption changes at ~425 mμ and ~530 mμ, however, are not reversible at – 150° C., that is, a product is trapped at – 150° C. (Only after thawing to 0° C. does a back reaction occur.) These results show that the trapped product is probably one of the two primary products (D^+ or A^-). The course of the negative absorption change as function of wave-length in the range of 425 mμ suggests that the primary product is probably identical with the oxidized form of a cytochrome ($D^+ = \text{cyt}^+$). The small positive change of absorption at ~530 mμ, however, normally does not appear on oxidation of cytochromes. It should be emphasized that the experiments have been carried out with material which was photochemically active after the temperature had been raised from –150° C. to +20° C. (proof by Hill reaction).

An irreversible oxidation of cytochrome at low temperatures in purple bacteria, especially *Chromatium*, has been already reported by Chance and Nishimura[6].

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Fixation of Nitrogen-15 by Excised Nodules of *Discaria toumatou*

Discaria toumatou (Rhamnaceae) has previously been recorded as a nodulated plant in New Zealand[1]. The nitrogen-fixing ability of its nodules has now been studied experimentally.

In these experiments seedlings of *D. toumatou* made no growth in aerated nutrient solutions lacking added nitrogen whether nodules were present or not. In solutions containing 5 p.p.m. nitrogen as nitrate only, plants that were nodulated grew normally, non-nodulated plants becoming moribund.

Nodulated plants of *Coriaria arborea*, in contrast, made normal growth in aerated nutrient solutions lacking added nitrogen, but became moribund when nodules were absent. Previous work has shown that excised nodules of this plant are able to fix atmospheric nitrogen[2].

Experiments in which excised nodules of *Discaria* were exposed to an atmosphere enriched with nitrogen-15 have also been carried out. Some nodules for these experiments were obtained from plants dug in the field. The roots were washed free of soil and transferred within 2 hr. to flasks containing an aerated nutrient solution lacking added nitrogen. They remained in this solution for two days before nodules were excised and exposed to nitrogen-15. Other nodules were obtained from plants of *Discaria* growing in aerated nutrient solution containing 5 p.p.m. nitrate nitrogen. Excised nodules of *Coriaria arborea* were exposed to nitrogen-15 at the same time. These were obtained from plants growing in aerated nutrient solutions as shown in Table 1.

All nodules were exposed for 40 hr., as previously described[2], to an atmosphere containing 90 per cent nitrogen enriched with 32 atoms per cent excess nitrogen-15. Nitrogen was then extracted with 3*N* hydrochloric acid.

Table 1. ENRICHMENT OF EXCISED NODULES FROM ATMOSPHERE CONTAINING NITROGEN-15

	Nitrogen-15 enrichment (atoms per cent excess)
Discaria from soil	0·10
Discaria from 5 p.p.m. nitrogen solution	0·095
Coriaria from solution lacking nitrogen	> 0·2
Coriaria from 5 p.p.m. nitrogen solution	0·22
Coriaria from 40 p.p.m. nitrogen solution	0·33

These experiments provide evidence that nodules of *Discaria toumatou* are capable of fixing atmospheric nitrogen. Under the conditions employed, however, they differed from those of *Coriaria arborea* in being unable to supply the plant's total need of nitrogen.

In the field *Discaria* grows quite differently from what it does in solutions: it is spiny and almost leafless. One would suppose that this type of growth uses less nitrogen, and it is therefore possible that the nodules can support the growth of *Discaria* in Nature and, perhaps, even that of its usual associates such as sweet briar (*Rosa rubiginosa*).

I am indebted to Mr. T. A. Rafter, director of the Institute of Nuclear Sciences, Department of Scientific and Industrial Research, and Dr. A. D. Campbell, Chemistry Department, University of Otago, for their collaboration in this work, which has been aided by a grant from the University of New Zealand research fund.

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BACTERIOLOGY

Lysogenic Conversion in the Genus *Proteus*

THE presence of prophage renders a bacterial cell immune to homologous bacteriophage. Homologous phage often adsorbs and penetrates the immune cell but does not undergo reduction to prophage and its genetic material is gradually 'diluted out' during multiplication of the cell[1]. Burnet and Lush[2] were the first to encounter a variation of this theme. They studied a lysogenic coccus which did not adsorb the homologous phage. Other workers[3,4] have since described systems in *Salmonella* where the presence of prophage modifies adsorption of homologous or heterologous phage. Changes in properties of bacteria occasioned by the presence of prophage are termed lysogenic conversions[5].

The recovery of a series of temperate phages from lysogenic strains of *Proteus* enabled the study of transduction[6] and conversion in this genus. A series of eleven different phages were employed. Phages 12/57, 49/*F*7, 50/*F*7 and 54/*F*7 were obtained by ultra-violet irradiation of *Proteus* strains 12, 49, 50 and 54, respectively. The indicator organism for the phage obtained from *Proteus* 12 was *Proteus* 57, and the indicator organism for phages from *Proteus* 49, 50 and 54 was *Proteus F*7. Phages 34/13 and 36/13 were present in the supernatant fluids of 10-day broth cultures of *Proteus* 34 and 36, respectively. *Proteus* 13 was the indicator organism for both these phages. Phages $\frac{6}{24}/6$, $\frac{12}{57}/57$, $\frac{14}{20}/14$, $\frac{14}{20}/20$ and $\frac{24}{25}/14$ were present in the supernatant fluids of 10-day broth cultures of *Proteus* strains 6 and 24, 12 and 57, 14 and 20, 14 and 20, 24 and 25, respectively. The indicator organisms for these phages were *Proteus* strains 6, 57, 14, 20 and 14, respectively. Organisms 49, 50 and 54 are *vulgaris* varieties of *Proteus*. The remainder are *mirabilis* strains.

Adsorption experiments of homologous phage to the lysogenic *A* phase[7] wild strains were done by adding 1 ml. of phage suspension to 1 ml. of an aerated overnight broth culture of the organism at a multiplicity of infection of 1. After 20 min. at 37° C. the bacteria were rapidly deposited by centrifuging and the clear supernatant titrated for unadsorbed phage. The 20 min. allowed for adsorption is less than the latent period of any of the phages and more than required for 95 per cent adsorption to indicator strains. Control experiments consisted of 1 ml. of the phage suspension in 1 ml. of broth. These were treated like the tests. Results indicate that only strain 54 adsorbs homologous phage (phage 54/*F*7). In this instance the phage titre of the supernatant was reduced from 1×10^9/ml. (control) to 1×10^6/ml. The remaining 10 phages do not adsorb at all to the corresponding wild lysogenic strains.

The lysogenic *Proteus* were irradiated with ultraviolet light according to the method of Lederberg and Lederberg[8]. Among the survivors of strains 12, 34, 36, 49, 50 and 54 colonies were recovered which are lysed by homologous phage. These sensitive variants are presumably organisms which have been cured of prophage as no phage can be recovered from them. Plaques formed by homologous phage on these sensitive variants have turbid centres due to the growth of lysogenized cells. Cultures of the turbid centres yield organisms with identical phage-adsorbing properties to those of the corresponding wild strain. Prophages present in wild strains 12, 34, 36, 49 and 50 are thus converted in respect of phage-adsorbing capacity. Although non-lysogenic derivatives of the hosts of phages $\frac{6}{24}/6$, $\frac{12}{57}/57$, $\frac{14}{20}/20$, $\frac{14}{20}/14$ and $\frac{24}{25}/14$ have not been obtained it is probable that these prophages exert a similar converting effect. Prophage 54/*F*7 is the exception. Lysogenic immune wild *Proteus* 54 adsorbs phage 54/*F*7 as readily as the sensitive variant.

Phages 12/57 and 34/13 previously used in transduction experiments[6] readily lysogenize most cells of the indicator strains (*Proteus* 57 and 13 respectively) at multiplicities of infection > 5. *Proteus* 57 and 13 lysogenized in this manner and labelled 57(12/57) and 13(34/13) respectively were freed of unadsorbed phage by suspending in the corresponding phage antiserum for 30 min. After centrifuging, the serum was decanted and the deposit streaked on MacConkey agar to yield single colonies. These colonies have the following properties:

(1) Broth cultures of 57(12/57) and 13(34/13) spontaneously liberate phage active on corresponding non-lysogenic strains. These phages are neutralized by antiserum to phages 12/57 and 34/13 respectively.

(2) The strains do not adsorb homologous phage.

(3) They are morphologically identical to strains 57 and 13.

(4) *Proteus* 13(34/13) does not adsorb phages 5 and 25. These phages were isolated from sewage and are active on *Proteus* strains 5 and 25 respectively[9]. *Proteus* 13 is lysed by these phages.

(5) Organisms 57(12/57) and 13(34/13) when matched against 57 and 13 respectively in the *A* phases[7] on nutrient agar show positive Dienes[10] phenomena, that is, they do not swarm into each other and maintain a clear line of demarcation. Fig. 1 illustrates this reaction between strains 57(12/57) and 57. The antigenic differences involved which are responsible for this phenomenon have not yet been identified.

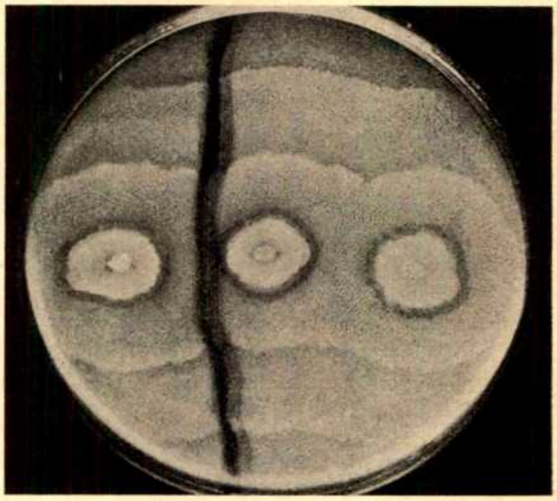

Fig. 1. Nutrient agar plate inoculated at one point on the left with strain 57(12/57) and at two adjacent points with strain 57. Incubated overnight at 37° C. Note how the inocula of strain 57 swarm into each other. The sharp line of demarcation between this spread and the swarming of strain 57(12/57) on the left denotes a positive Dienes phenomenon

Strains 57 and 13 can be transduced to streptomycin resistance by phages 12/57 and 34/13 propagated on streptomycin-resistant mutants of 57 and 13 respectively[4]. Lysogenic transductants possess all the properties of strains lysogenized with non-transducing phage.

Differences in properties of strains 57 and 57(12/57) and strains 13 and 13(34/13) are attributed to the presence of converting prophages 12/57 and 34/13. Conversion is in respect of phage-adsorbing capacity and possibly other antigenic changes as well.

Phages 12/57 and 34/13 are thus competent in transducing and converting systems. A similar position obtains with *Salmonella* phage *P*22. This phage has been extensively used in transduction experiments and can produce lysogenic conversion[4]. A possible difference is that this phage is capable of producing conversion in the vegetative state (phage conversion). No attempt has yet been made to investigate the latter possibility for the *Proteus* phages mentioned.

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Elimination of Transmissible Drug-resistance by Treatment with Acriflavin

The first isolation of multiple resistant *Shigella* was reported by Kitamoto[1]. This was resistant to four drugs (r_4): streptomycin, tetracycline, chloramphenicol and sulphanilamide. *E. coli* r_4 was isolated in the epidemic of *Sh. flexneri* 3*a* r_4 (refs. 2 and 3). *Sh. flexneri* 2*a* r_3 and *E. coli* r_3 were also isolated in another epidemic, which were resistant to streptomycin, chloramphenicol and sulphanilamide[4]. *Citrobacter* r_4 and *E. coli* r_4 were isolated from a dysenteric patient after treatment with chloramphenicol[5].

From the epidemiological investigation of the multiple resistant *Escherichia* in the fæces of human beings[6], we have learned more about the multiple resistant *Shigella* and the general phenomena among Enterobacteriaceae.

Ochiai[7] and Akiba[8] reported that the multiple resistance was transmitted between *Shigella* and *E. coli* following mixed cultivation. This transmission is not mediated by transduction, transformation or filtrable agent, but by cell-to-cell contact[9,10] without regard to the polarity of *F* factor[11]. It has also been shown that the multiple resistance is transmitted between every genera of Enterobacteriaceae[12], whereas perhaps only 3–5 per cent of the strains of *Escherichia* would have given positive results in bacterial recombination.

Artificial elimination of the transmissible drug-resistance by treatment with acriflavin was carried out by Hirota's method[13].

The drug-resistant cells were inoculated into Difco brain-heart infusion broth containing sub-lethal concentrations of acriflavin (20 μgm./ml. for *E. coli* and 10 μgm./ml. for *Shigella*). After overnight incubation at 37° C., the cells were streaked on Drigalski's medium and the drug-resistance of each colony was tested. Heart infusion agar was used for assaying resistance to streptomycin, chloramphenicol and tetracycline. For assaying sulphanilamide resistance, semi-synthetic medium was used. It consists of 1 litre of medium *A* (ref. 14) agar enriched with 2·0 gm. casamino-acid, 10 mgm. tryptophan, 2 mgm. nicotinic acid, 10 mgm. thiamin hydrochloride and 2 gm. glucose. The results are shown in Table 1.

Table 1. FREQUENCY OF ELIMINATION OF DRUG-RESISTANCE BY TREATMENT WITH ACRIFLAVIN

Micro-organisms	With acriflavin treatment	Without acriflavin treatment
E. coli O–26 r_4	5/579 (0·9 %)	0/1,589*
,, r_3	15/547 (2·9 %)	0/881
,, r_1	0/579	0/364
Sh. flexneri 3*a* r_4	241/517 (46·6%)	0/1,198
,, r_3	0/375	0/1,177
,, r_1	201/583 (36·2 %)	0/1,116

r_4, resistant to streptomycin, chloramphenicol, tetracycline and sulphanilamide; r_3, resistant to streptomycin, tetracycline and sulphanilamide; r_1, resistant to tetracycline.

* Numerator, number of the drug-sensitive colony; denominator, number of the total colonies tested.

The drug-resistance of both *Escherichia* and *Shigella* were eliminated from the drug-resistant cells after treatment with acriflavin, and the frequency of elimination of the drug-resistance was higher in *Shigella* than in *Escherichia*. This fact is in agreement with the finding that the loss of the drug-resistance with *Shigella* was more frequent than with *Escherichia* after 6 months storage in cooked meat media. It was also found that all markers for drug-resistance were lost by treatment with acriflavin and the cells became sensitive to all drugs. The drug-sensitive cells thus obtained were not able to transmit the drug-resistance by mixed cultivation with other drug-sensitive cells.

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GENETICS

Structural Mutations

THE premise that linear and continuous sequences of nucleotides in the genetic material (deoxyribonucleic acid (DNA) in most organisms, ribonucleic acid (RNA) in some viruses) specify corresponding linear and continuous sequences of amino-acids in the proteins, that is, the gene products of these organisms, is so fundamental to present-day thinking in the field of molecular biology that it has rightfully been referred to as the 'central dogma'[1,2]. Another premise, inherent in this dogma, is the principle of colinearity of the genetic map with the nucleotide sequence and the definition of a mutation as an alteration in this sequence giving rise in turn to an altered structure in the product proteins. The conclusion has therefore been implicit that every mutation must necessarily result in some alteration in a gene product.

It is the purpose of this communication to point out that this syllogism is neither logically sound nor necessarily reflective of the situation existing in Nature even on the basis of the fragmentary evidence at present available. It is conceivable that there exist regions in the genetic nucleic acid primarily concerned in the specification not of gene products but with nucleic acid structure itself. The structure here referred to is a 'tertiary' folding or other configurational alteration superimposed on the 'secondary' structure (the hydrogen-bonded helix). That such folding must exist in native genetic material is self-evident from a consideration of the dimensions of the heads of bacteriophages $T2$ or $T4$ (95×65 mμ)[3], as contrasted with those of the DNA contained therein were it to assume the fully extended, rigid, configuration demanded by the Watson–Crick hypothesis ($6 \cdot 3 \times 10^4$ mμ $\times$ 2 mμ). We[4,5] and others[6,7] have speculated that the secondary constituents associated with the DNA of these phages (internal protein(s)[7,8], polyamines[6,9]) may be concerned with the attainment and/or maintenance of this tertiary structure. The hypothesis of scattered regions concerned with tertiary structure has, however, other important consequences which can be tested.

It may be fruitful, for example, to institute a search for phage mutants, which possess *solely* an altered DNA, as a consequence of which they differ in their tertiary structure and in their DNA-protein interaction without, however, any significant alteration in the primary structure of any of the individual proteins specified by this DNA. It is possible that in $T2$ and/or $T4$ at least such mutations may already have been reported. One such might be the well-known class of acridine-resistant mutants[10,11]. On the basis of work by others[6,12–16] and investigations now in progress in our laboratories, it is likely that, in the dark under carefully controlled conditions, acridines in general and proflavine in particular exert a very specific inhibitory action on phage reproduction. This action occurs during that stage of maturation which is concerned with the integration of previously synthesized, structurally intact, and fully functional DNA with the structural phage proteins, mainly those of the head. The dye has no effect on the elaboration of either DNA (refs. 6, 15) or proteins[14,15] separately. The inhibitory effect may find its cause in the formation of a relatively strong and specific complex between the dye and phage DNA which is, however, rapidly and reversibly dissociated by dilution in the presence of normal phage constituents. Four predictions can then be made: (1) that a one-step proflavine-resistant mutant should be one possessing an altered DNA but normal proteins which allows maturation to proceed at concentrations of dye completely inhibitory to the wild type; (2) that it should be possible to detect this alteration in the DNA by comparing interactions of dye and DNA's from wild-type and mutant respectively by appropriate physical and chemical techniques, while phage proteins isolated from mutant and wild type should be identical in structure and indistinguishable by all criteria; (3) that a relatively large number of different mutants of this type should be isolatable and that these markers should be distributed more or less at random along the full length of the phage genome; (4) that multi-step mutants, which are resistant to higher levels of dyes, should manifest enhanced resistance by the criteria enumerated under (1) and (2).

Prediction (3) brings to mind another class of mutants. The *ht* (extended host range, turbid) markers studied and mapped very carefully by Baylor and her collaborators[16] appear to possess just such a distribution. Yet recent genetic and immunochemical work by Streisinger[17] and structural investigations by Williams and Fraser[18] make it apparent that, phenotypically, host-range and attachment to cells are manifested through just one species of protein, that of the tail fibres[19]. Once again those mutations may control not the structure of a protein *per se* but only that of the nucleic acid itself: in turn this structural alteration controls the interaction of the DNA with some protein(s), itself specified by quite a different section of the genome. Indications of this may be provided by Baylor's recent work on the stepwise and additive nature of these *ht* mutations with respect to their stability to incubation in buffer (Baylor, M. B., private communication).

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, March 20

INSTITUTION OF ELECTRICAL ENGINEERS, LONDON GRADUATE AND STUDENT SECTION (joint meeting with the Utilization Section, at Savoy Place, London, W.C.2), at 6 p.m.—Mr. P. J. H. Rata: "Aluminium in Electrical Engineering"; Mr. A. D. Swanston and Mr. B. W. Towell: "Diesel Railway Locomotives"; Mr. T. E. S. Rickard: "Automatic Programmes for Hot Reversing Mills".

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Miss Margaret F. Harker: "Photography in Education" (Cantor Lecture).

INSTITUTE OF METAL FINISHING (at Northampton College of Technology, St. John Street, London, E.C.1), at 6.15 p.m.—Mr. U. F. Marx and Mr. D. Povey: "Zinc Plating from the Pyrophosphate Bath".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Ewell County Technical College Faraday Society, at the Ewell County Technical College, Reigate Road, Ewell, Surrey), at 7 p.m.—Dr. R. N. Haszeldine: "The Chemistry of Fluorine".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Sir William Holford: "Birth of a City".

Monday, March 20—Tuesday, March 21

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 10 a.m. daily—Symposium on "Fungicides in Agriculture and Horticulture".

Tuesday, March 21

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Discussion meeting on: "Some Factors in the Safe Operation of Air Separation Plants".

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Self-Adaptive Control Systems" opened by Dr. J. H. Westcott, Dr. J. L. Douce, Dr. P. E. W. Grinsted and Mr. P. H. Hammond.

UNIVERSITY OF LONDON (at the Senate House, University of London, W.C.1), at 5.30 p.m.—Prof. W. Z. Hassid (University of California): "Transformation of Carbohydrates in Plants".*

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. D. A. Wright: "Thermoelectric Generation".* (Last of five lectures on "Generation of Electricity without the use of Rotating Machinery".)

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. G. H. Lee: "Some Aspects of Buffeting".

Tuesday, March 21—Thursday, March 23

INSTITUTE OF METALS (at Church House, Great Smith Street, *London, S.W.1), at 10 a.m. daily—Spring Meeting.*

Wednesday, March 22

INSTITUTE OF PHYSICS AND THE PHYSICAL SOCIETY, COLOUR GROUP (in the Lecture Theatre of the British Lighting Council, Lancaster Place, London, W.C.2), at 3 p.m.—Mr. H. L. Gloag: "Some Principles of Good Colour in Buildings"; Mr. D. L. Medd: "Influences on the Use of Colour in Building in the United States of America".

GEOLOGICAL SOCIETY OF LONDON (at Burlington House, Piccadilly London, W.1), at 5 p.m.—Prof. F. Hodson and Dr. G. C. Lewarne: "A Mid-Carboniferous (Namurian) Basin in Parts of the Counties of Limerick and Clare, Ireland".

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Discussion on "The Future of High Speed Storage Systems".

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Mr. E. Holmes-Smith, Mr. J. Lister and Mr. P. V. Liddell: "The New Wilton High-Pressure Cyclone Boiler".*

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND SUPPLY SECTIONS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. Carruthers and Mr. D. L. Smart: "The Engineering Aspects of Plasma Physics".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Sir Frederick Bartlett: "The Bearing of Psychology and Medicine upon Engineering" (James Clayton Lecture).

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Mr. J. Mauron: "Control of the Nutritional Qualities of Milk Protein".

Thursday, March 23

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Monsieur J. Prevost: "French Biological Work in the Region of Isle Kerguelen".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 6 p.m.—General H. Ziegler: "Problems of Short Take-off and Landing". (Fourteenth Louis Blériot Memorial Lecture.)

OIL AND COLOUR CHEMISTS' ASSOCIATION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. H. C. Shellard: "Can the Climatologist *Help You?*"

Friday, March 24

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Geophysical Discussion on "Geodetic Uses of Artificial Satellites". Chairman: Dr. A. H. Cook. Speakers: Brigadier G. Bomford, Dr. R. d'E. Atkinson, Dr. A. P. Willmore and Mr. W. H. Guier.*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 25).

ASSISTANT LECTURER (trained in electron microscope techniques) IN BIOPHYSICS in the Faculty of Medicine—The Registrar, The University, Manchester 13 (March 25).

PRINCIPAL (graduate of a British university, responsible experience in technical education, and preferably some industrial experience) OF THE FROME TECHNICAL INSTITUTE—Chief Education Officer, County Education Office, Trull Road, Taunton, Somerset (March 30).

HEAD OF THE DEPARTMENT OF MATHEMATICS—The Principal, Northampton College, St. John Street, London, E.C.1 (March 31).

IMPERIAL CHEMICAL INDUSTRIES FELLOWS (preferably with the degree of Ph.D. or equivalent research experience) to carry out original research in chemistry or physics, for a period not exceeding three years—The Registrar, University College of North Staffordshire, Keele, Staffs (March 31).

ASSISTANT LECTURER or LECTURER (preferably with an honours degree in mathematics) for work at the University Tidal Institute and Observatory—The Registrar, The University, Liverpool (April 4).

ASSISTANT LECTURER or LECTURER (with an honours degree in physical oceanography or physics, and prepared to spend several weeks each year at sea) IN THE DEPARTMENT OF OCEANOGRAPHY—The Registrar, The University, Liverpool (April 4).

LECTURER or ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Leeds (April 4).

LECTURER (with a good honours degree, teaching experience up to degree level, and research experience) IN ZOOLOGY at the Nigerian College of Arts, Science and Technology, Zaria Branch—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (April 7).

RESEARCH BIOCHEMIST (with postgraduate research experience in the fields of protein chemistry or nutrition), to join a team investigating the effects of heat treatment on nutritional availability of the amino-acids in the proteins of foods—The Secretary, School of Agriculture, The University, Downing Street, Cambridge (April 7).

SENIOR LECTURER IN PHARMACY in the Science Department, Jamaica College of Arts, Science and Technology, Kingston—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (April 7).

LECTURER (with special qualifications in Latin American geography) IN GEOGRAPHY—The Registrar, The University, Leicester (April 8).

CHAIR OF PHYSICS—The Registrar, The University, Sheffield (April 15).

ASSISTANT LECTURER (with an honours degree in mathematics and preferably an interest in numerical analysis) IN MATHEMATICS—The Registrar, King's College (University of London), Strand, London, W.C.2 (April 17).

READER IN PHYSIOLOGY at London Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 17).

LECTURER IN PHYSICS at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 30).

LECTURER IN THE DEPARTMENT OF PHYSIOLOGY at Makerere University College, East Africa—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 30).

SENIOR LECTURER IN PHILOSOPHY at the University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 30).

UNIVERSITY ASSISTANT LECTURER IN ARCHÆOLOGY to give instruction in the archæology of the Anglo-Saxon and Celtic peoples—The Secretary of the Appointments Committee, Faculty of Archæology and Anthropology, Downing Street, Cambridge (May 15).

ASSISTANT OR ASSOCIATE PROFESSOR (with a Ph.D. and teaching experience) IN PHARMACOLOGY—Dr. H. G. Downie, Professor and Head, Department of Physiological Sciences, Ontario Veterinary College, Guelph, Ontario, Canada.

HEAD (physical chemist with outstanding qualifications and experience, and an original outlook) OF THE PHYSICO CHEMICAL DIVISION, to direct basic studies into the bulk properties of polymers and to initiate research in his own field of interest—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian.

LECTURER IN PHYSICS—The Clerk to the Governors, Northern Polytechnic, Holloway, London, N.7.

SENIOR TECHNICIAN (with experience in a physiological laboratory) to assist in work on lung function—The Secretary, Institute of Child Health, The Hospital for Sick Children, Great Ormond Street, London, W.C.1.

UNION CARBIDE POSTDOCTORAL FELLOW IN ORGANIC CHEMISTRY—Prof. R. C. Cookson, The University, Southampton.

REPORTS and other PUBLICATIONS

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

General Register Office. Studies on Medical and Population Subjects, No. 16: Area of Residence of Mental Hospital Patients—Admissions to Mental Hospitals in England and Wales in 1957 according to Area of Residence, Diagnosis, Sex and Age. Pp. iv+177. (London: H.M. Stationery Office, 1960.) 15*s*. 6*d*. net. [1312

Department of Scientific and Industrial Research. The Human Implications of Work Study—The Case of Pakitt, Ltd. By Stuart Dalziel and Lisl Klein. Pp. xiii+81. (Stevenage: Human Sciences Unit, D.S.I.R., Warren Springs Laboratory, 1960.) [1312

Manchester Literary and Philosophical Society. Memoirs and Proceedings, Vol. 102, 1959-60. Pp. 81+xliii. (Manchester: Manchester Literary and Philosophical Society, 1960.) 31*s*. 6*d*. [1312

Hatfield Technical College. Eighth Annual Report, Session 1959-60. Pp. 44. (Hatfield: Hatfield Technical College, 1960.) [1312

Biology, Psychology and Belief. By Dr. W. H. Thorpe. (The Fourteenth Arthur Stanley Eddington Memorial Lecture, 3 November 1960.) Pp. 60. (Cambridge: At the University Press, 1961.) 4*s*. 6*d*. net. [1312

Air Ministry: Meteorological Office. British Rainfall 1957: Report on the Distribution of Rain in Space and Time over Great Britain and Northern Ireland during the year 1957 as recorded by about 5,000 Observers. (Ninety-seventh Annual Volume). Pp. iv+245. (M.O. 663). (London: H.M. Stationery Office, 1960.) 32*s*. 6*d*. net. [1312

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 83: Management and Feeding of Poultry Breeding Stock. Pp. 11. (Belfast: Ministry of Agriculture, 1960.) [1312

Proceedings of the Royal Irish Academy. Vol. 61, Section A, No. 1: The Absolute Convergence of Certain Lacunary Fourier Series. By Siobhan O'Shea. Pp. 1-8. 1*s*. 6*d*. Vol. 61, Section B, No. 7: A Study of the Caudal Gills of Dragonfly Larvae of the Sub-order Zygoptera. By Niall MacNeill. Pp. 115-140+plates 5-9. 5*s*. Vol. 61, Section B, No. 8: The Relationships of some Bog Communities in Western Galway. By D. J. Boatman. Pp. 141-166+plates 10 and 11. 4*s*. 6*d*. Vol. 61, Section B, No. 9: Observations of the Serum Proteins of the Bass *Morone labrax* (L.) (Pisces: Serranidae). By Fergus J. O'Rourke. Pp. 167-176. 1*s*. 6*d*. Vol. 61, Section B, No. 10: The Dragonflies of the Gearagh, West Cork. By Ann Hewitson and Fergus J. O'Rourke. Pp. 177-185. 1*s*. (Dublin: Hodges, Figgis and Co., Ltd., 1960.) [1312

Tube Investments, Ltd. This is T.I. Today. Pp. 16. (London: Tube Investments, Ltd., 1960.) [1312

Planning, Vol. 26, No. 447 (12 December 1960): Community Mental Health Services. Pp. 337-356. (London: Political and Economic Planning, 1960.) 2*s*. 6*d*. [1312

Ministry of Aviation. Reports on Plastics in the Tropics No. 13: Polystyrene Mouldings. Pp. 42. (London: H.M. Stationery Office, 1960.) 5*s*. net. [1312

The Nature Conservancy. Report of the Nature Conservancy for the year ended 30th September 1960. Pp. vii+138+14 plates. (London: H.M. Stationery Office, 1960.) 6*s*. 6*d*. net. [1312

Imperial College of Science and Technology (University of London). Fifty-third Annual Report of the Governing Body, 1959-1960. Pp. iv+123. (London: Imperial College of Science and Technology, 1960.) [1312

The Institute of Physics and the Physical Society. Handbook of Scientific Instruments and Apparatus, 1961 Exhibition. (Advertisement Section for Exhibition Handbook.) Pp. 75. (London: Institute of Physics and Physical Society, 1960.) [1312

Contemporary Psychology and Christian Belief and Experience. By Prof. Malcolm A. Jeeves. Pp. 16. (London: The Tyndale Press, 1960. Published for the Research Scientists' Christian Fellowship.) 1*s*. 6*d*. [1312

National Park Guides, No. 3: Peak District. Pp. viii+90+26 plates. (London: H.M. Stationery Office, 1960.) 5*s*. net. [1312

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THE UNDER-DEVELOPED TERRITORIES OF THE WORLD

IT is not surprising that in the debate in the House of Lords on January 25 on the North Atlantic Treaty Organization reference should be made to the recent report, *Increasing the Effectiveness of Western Science* (see *Nature*, 188, 879 ; 1960), and particularly to the proposal to create an Atlantic Institute of Science and Technology, a proposal which was strongly supported by Earl Jellicoe. Although Lord Carrington, who replied on the debate for the Government, agreed that the proposal merited serious consideration, he declined to commit the Government until the working group, on which Sir John Cockcroft is the United Kingdom member, had reported. While, however, there was other support in the debate, particularly from Lord Gladwyn, the Baroness Elliot and the Earl of Listowel, for the work which the newly formed Organization of Economic Co-ordination and Development of the North Atlantic Treaty Organization could do in co-ordinating all scientific activity, including the encouragement of the production of scientists and technologists, a notable, if not surprising, feature of the debate was the way in which these activities were related to the task of helping the under-developed territories.

This was seen as a moral responsibility, an important aspect of economic policy, and an essential element in the effective defence of the Atlantic Community. The Earl of Listowel, for example, welcomed the establishment of this new Organization as bringing Canada and the United States for the first time into full economic co-operation with other countries of the NATO group in Western Europe. This would facilitate the prior consultation and co-ordination of trade and economic policies that are essential if the West is to do more for the development of the under-developed countries, which still receive most of their capital investment and technical skill from the West. Lord Ogmore, in opening the debate, had stressed the need to increase assistance to the under-developed countries, and it was a key *motif* of the debate that technical assistance is an essential aspect of Western defence policy and in countering the social and economic challenge of Communism in those countries.

It is remarkable, therefore, that this theme should be absent when the Overseas Service Bill received its second reading in the House of Commons on January 24. The Bill was generally welcomed in the debate, and in moving the second reading the Colonial Secretary, Mr. Iain Macleod, stressed that when the Bill became law there would be a three-point structure in the Service, one of which was a vast scheme of technical assistance ; nevertheless, the debate generally was concerned rather with the other two points—the schemes of compensation and the public officers' agreement. Mr. Macleod could not add anything to his announcement in December that the Government is studying the establishment of a department or a ministry of technical aid or assistance, except that he hoped the results of this study would be available in a few weeks ; though he remarked that he regarded the Bill, which put heavy expenditure on the British taxpayer, as the most important proposal in relation to technical aid for the under-developed countries of the Commonwealth to be put before the House since the Colonial Development and Welfare Act.

Mr. Marquand, for the Opposition, agreed that the Bill contains far-reaching proposals, potentially of great value to the emerging countries, especially within the next five or ten crucial years of their development, and he thought that the implementation of its provisions would contribute to a world-wide extension of technical assistance and expert aid in the provision of the essential structure of development—the improvement of education, public health, agriculture, etc. Mr. P. Wall expressed the hope that Britain would go further than the Ministry for Overseas Aid which Mr. Macleod is at present considering, and endeavour to lay a foundation for the creation of an Overseas Service with interchangeable posting, to serve foreign countries as well as Commonwealth countries and provide administrators and technologists for United Nations organizations and for technical aid throughout the world. He believes there is a great future in this idea, and that development along such lines would ensure that the British genius for administration is not lost to the world.

Dr. Barnett Stross strongly supported the same idea, and, while concerned primarily with medical aid, suggested the creation of a Commonwealth Advisory Council to advise the Minister on medical and technical assistance generally. These suggestions received no comment from Mr. H. Fraser, the Under-Secretary of State for the Colonies, in replying on the debate. Although the debate was indeed concerned largely with the way in which the provisions of the Bill would affect existing officers of the Service, the importance of the contribution which Britain is making to technical assistance was fully recognized, and even the magnitude of the financial obligations which Britain will assume under the Bill was unchallenged except by Sir Kenneth Pickthorn. Mr. Macleod estimated the financial cost at possibly £150 million during the next ten years, and when to this are added the contributions under the Colonial Development and Welfare Acts of more than £25·5 million a year and of bilateral and multilateral assistance of roughly £140 million a year in 1959–60, the magnitude of Britain's effort will be appreciated.

The financial contribution, however, is only one aspect. The demand made on the scientific and technical resources of Britain, particularly in man-power, has also to be considered, and it is in this respect that the last report on *Colonial Research* was so disappointing. The Council for Scientific and Industrial Research, the Nature Conservancy and the Agricultural Research Council, in addition to the

Overseas Research Council, make important contributions to research overseas in various fields ; but there was no evidence in that report that the Overseas Research Council is attempting to provide a conspectus of the overall effort and the resources available. All the research councils are responsible to the Minister for Science, but the report provided no evidence that the Overseas Research Council is, in fact, doing anything that the Advisory Council on Scientific Policy could not do, or even to justify the change of name from the Colonial Research Council.

Difficult as they may be, the questions of co-ordination of effort and of communication are increasingly important, and it is as well to note that last year the Overseas Development Institute was formed to serve as a centre to which all those interested in the problems of developing countries could turn for information about the needs of those countries, and the action that is being taken to meet them. The Institute is intended to become a central office of information of this field generally, and also to serve as a centre in which those interested in particular aspects of these problems can meet and exchange views. Besides helping to make people dealing with such problems in government, academic, industrial or commercial undertakings aware of each other's activities, and to bring together those in the United Kingdom who are concerned with these problems and those who, in the developing countries themselves, have intimate knowledge of them, the Institute is intended to keep the public informed of what is going on in this field, of the needs of the developing countries and of what the industrialized countries can and should do to help.

The Institute is being financed by the Ford Foundation and by contributions from industrial and commercial undertakings in the United Kingdom, and it clearly envisages a wider field than the Overseas Research Council or even the Advisory Council on Scientific Policy could be expected to cover. Nevertheless, its formation points to a matter to which the Minister for Science might well give some attention and which should not be overlooked in the study now being made of the most appropriate means of channelling technical aid. The scale on which Great Britain is now contributing, financially and in trained man-power, to overseas development and welfare in the technical and scientific fields makes it imperative that that effort should be effectively co-ordinated and related realistically to the resources of the country, and to its other commitments and demands, as well as to the needs of the under-developed territories.

Particular reference was made to the Overseas Development Institute by Prof. P. M. S. Blackett in his Hinchley Memorial Lecture to the Institution of Chemical Engineers on February 15. Discussing "Science and Technology and the Developing Countries", he returned to a main theme of his presidential address to the British Association just over three years ago, and particularly to aspects of the general problem which were the subject of the symposium on "World Food and Population" in the Association's meeting at Cardiff last September, on which indeed Prof. Blackett drew considerably. It is essential, he said, in the first place, that a young and poor country, setting out on the difficult road of rapid social, economic and technological development, should have a clear idea of what are the right priorities for its investments ; and, like Lord Fleck at Cardiff, he pointed out that the under-developed countries must concentrate on a higher yield per acre, rather than, as in Western agriculture, on greater output per man. Until there is plenty of alternative employment in the factories, the saving of agricultural labour creates more problems than it solves ; in most parts of Asia the problem is really to devise new technologies which make man-power more efficient without much capital expenditure.

Since the improvement of agricultural yields is the most vital single step in much of South-East Asia, and in particular in India, and because the chemical engineer produces the plant required for the manufacture of the essential fertilizers, pesticides and selective weed-killers, Prof. Blackett considered this aspect of agriculture in the under-developed countries in some detail. The problem is fundamentally that of the application of known methods of production of chemical aids to agriculture and their use ; this involves much in the way of large-scale field-trials and experimental farms. Prof. Blackett also suggested that the resources of modern technology could be used to design plant, such as that for the fixation of nitrogen, which is economically more suitable for an under-developed country short of capital but with abundant labour. Detailed technological–economic studies of the possibilities of plants of various sizes ranging down to a small plant with the lowest possible capital cost might be very rewarding ; and if any could be brought below £70 per ton a year they might be of great value. The rate of growth of Indian fertilizer production is primarily limited not by lack of scientific knowledge or labour, but by lack of capital.

There is a further factor to consider. While the cost of the optimum size of plant is in the region of £30 million, in general, central governments alone can sponsor them. If, however, the cost could be reduced to £1 million or less, in some circumstances they might come within the scope of State or local governments, or even of private native enterprise ; this would be in line with the tendency for increasing stress to be placed on the development of moderate-scale industry right down to the village level. Further, the rapid spreading of technology in this way throughout a country has an important bearing on technical education, and Prof. Blackett suggested that the training given to the operatives could make a vital contribution towards raising the level of technical education.

Putting his theme into more general terms, Prof. Blackett advocated much more study by Western industrialists of the requirements of the developing countries for all kinds of production goods, from large- and small-scale plants to simple tools, taking account both of the latest technical developments and of the specific economic and social conditions of these countries. Further, he suggested that Western firms operating in under-developed countries should deliberately plan their educational or training facilities in excess of their own needs as a considered contribution to the general technical education of the country in which a particular firm is operating. This he intends should apply to the training of native personnel both in their own country and in the United Kingdom ; and from the trend of Mr. Anthony Tuke's remarks, in his chairman's address at the recent annual general meeting of Barclays Bank, on the needs of under-developed countries and the importance of contributing the investment which they require, it would seem that some such response may increasingly be expected.

There is, moreover, strong support to be found for Prof. Blackett's views in the presidential address which Prof. Abdus Salam delivered to the Pakistan Science Congress at Dacca on January 11. Prof. Salam, who referred to Prof. Blackett's address at Dublin in 1957, began by suggesting that acquisition of the requisite technological skills and investment of at least 5 per cent of the national income in the appropriate productive enterprises are the major pre-requisites of self-reinforcing growth. Japanese experience, he said, has demonstrated that the necessary technology is easy to acquire ; Russian experience that priorities on heavy industry accelerate the growth, and Chinese experience that labour is itself a form of capital. So far as Pakistan is concerned, Prof. Salam urged that a political group determined to eradicate poverty is a third prerequisite, and he thought that the new five-year plan reflects the emergence of such a group. The plan recognized the need for bold planning for agricultural development, for exploiting minerals and for heavy industry, and also that liberal provision for developing technological and scientific skills is the wisest investment a nation can make.

The plan aims at a 10–15 per cent capital investment, of which 3–4 per cent is required in foreign exchange and must come from the advanced countries in the form of long-term loans or gifts, and Prof. Salam readily acknowledged that this has involved real sacrifice on the part of those countries. The plan envisages the establishment of heavy industry, particularly steel, with an output of 400,000 tons, and the manufacture in place of import of fertilizers. The major effort, however, is in the provision of the necessary technical skills, including provision for an output of 7,000 technicians a year. Prof. Salam believes that 1961 may mark the beginning of a new era in Pakistan in respect of scientific research and training programmes. He recognized that the success of the Plan depends on some factors beyond Pakistan's own control, but Pakistan's scientists, he said, can none the less make an immediate and essential contribution to the elimination of poverty in Pakistan.

Fully as significant, however, as this consensus of opinion as to the needs and possibilities of technical aid, is the extent to which the whole problem is now being discussed objectively. Mr. A. Schonfield's lucid and comprehensive analysis, accompanied by some provocative suggestions, in his book, *The Attack on World Poverty*, published last year, dealt indeed with one particular aspect and stressed primarily the part that the United Nations should play. Although support for his views may have been somewhat shaken by subsequent developments in the Congo, his arguments as to the need for some re-organization, and in fact for effective co-ordination of the work of the numerous agencies, in spite of the difficulties involved, have been strengthened independently of the soundness of Mr. Schonfield's particular suggestions.

Perhaps even more significant, however, is the discussion of this problem in an article, "The Hungry Two-thirds", which appeared in the *Westminster Bank Review* of November, while earlier in the year the American Academy of Political and Social Sciences devoted the May issue of its *Annals* to a symposium of papers dealing with "International Co-operation for Social Welfare—A New Reality", a feature of which was the stress laid on the need for such technical assistance to be given on altruistic grounds and not in any spirit of competition or as part of a strategy in the defence of the Western world. This point was made again in an article by E. Rabinowitch, "The Atomic Age Doctrine : Common Efforts for Common Aims", which appeared in the *Bulletin of the Atomic Scientists* of September, and the subsequent issue of the same *Bulletin* contained an article by E. S. Prentice, "Significant Issues in Economic Aid for the Developing Countries", which summarized a report of the Stanford Research Institute on that subject. A second article in the same issue (October), by P. T. Bauer, on "Economic Development of Under-developed Countries" was at pains to stress the complexity of the whole problem and the vital importance of the institutional or administrative framework which governments alone can ensure for economic activities.

The need for some sort of master plan to co-ordinate the various types of aid being given to the developing countries, which now runs to something like £1,900 million a year, is one of the points made in the *Westminster Bank Review* article—and the need to diagnose thoroughly the problems peculiar to each country ; and it is also recognized that to increase the flow of international aid to the minimum of £1,000 million a year during the next ten years, which P. Hoffmann estimates as required from the West alone over and above the existing level, is not a complete answer. A steadier level of industrial demand for raw materials would of itself enable the developing countries to help themselves more easily. The emphasis in this article is on the political, economic and humanitarian reasons for overseas aid to the developing countries, but it nevertheless lends a good deal of support in general terms to the argument of Prof. Blackett and of Prof. Salam. Incidentally, it also stresses the desirability of channelling such assistance, so far as is possible, through international agencies, and it may be recalled that the World Federation of United

Nations Associations last year renewed its campaign for a ten-year international programme of world economic development.

Ideally, international distribution of technical and other forms of assistance is the right solution; but the objective appraisals of the situation now being made cannot ignore the plain fact that much of the aid distributed in recent years has been wasted, and even without the recent shocks to confidence dealt by events in Africa, the United Nations in its present structure can scarcely be regarded as competent to handle efficiently the flow of development capital and technical assistance on the scale now contemplated. Moreover, in any re-organization, the difficult problems of world government and the sacrifices of national sovereignty involved can scarcely be ignored, nor is it easy to find any recent grounds for supposing that many of the World Powers are prepared to make such sacrifices, however small. Nevertheless, these articles alone show that, at the technical level, the problems are being faced objectively, and President Kennedy's speeches have at least indicated that increasing economic assistance to the less-developed countries will be a central theme of foreign policy under the new American Administration. The United States can scarcely do otherwise than make some effort to ensure that its effort is effectively and efficiently channelled, nor are the voluntary agencies and foundations, like the Rockefeller Foundation, the Ford Foundation and the Carnegie Corporation, to the important contributions of which Dr. L. M. Hoskins directs attention in his contribution to the *Annals of the American Academy of Political and Social Science*, likely to be indifferent to this aspect.

Ultimately, the organization by which overseas aid is distributed, whether technical or financial, must be a matter for determination by governments, and that applies alike to the United Nations and elsewhere. It is not an easy problem to solve, nor is clear and constructive thinking the only condition of success. Widespread public understanding of the issues and the reasons for the sacrifices involved is no less imperative, and for that reason alone, the objective discussion of overseas aid in all its aspects cannot be too wide-spread. With special aspects scientists and technologists have their own particular and direct concern—Dr. E. F. Witte discussed the development of professional leadership in his contribution to the American Academy's symposium—and it is important that they should see that all the implications of such demands on the limited scientific and technical resources and man-power of Britain are fully and clearly understood; the general problem is one to which as citizens they can scarcely be indifferent. While their first concern may well be with the scientific and technical aspects, they have no less the opportunity and responsibility to see that the whole problem is objectively discussed, and to ensure that all the implications of aid on the scale now required, more especially in respect of international organization and administration, are fairly faced and resolutely and honestly handled.

CLASSICAL PHYSICS AND MODERN TECHNOLOGY

Physical Methods of Organic Chemistry

Part 1. Edited by Arnold Weissberger. (Technique of Organic Chemistry, Vol. 1, Part 1.) Third completely revised and augmented edition. Pp. xii + 918.

Part 2. Edited by Arnold Weissberger. (Technique of Organic Chemistry, Vol. 1, Part 2.) Third completely revised and augmented edition. Pp. xii + 895–1797 + 21.

(New York: Interscience Publishers, Inc.; London: Interscience Publishers, Ltd., 1959, 1960.) 184*s*. each.

IT was said, some forty years ago, of a prominent teacher of the then rapidly expanding subject of physical chemistry that he would attract to his laboratory many enthusiastic students by the epigram: "What you have called physics we here call chemistry". The forefronts of research in physics and chemistry have, since then, diverged, but it is evident to-day that twentieth-century chemistry has engulfed nineteenth century physics, for chemists and chemical technologists now make extensive use of exact measurements of the physical properties of different forms of matter.

With the widening of the scope of chemical science and the rapidity of the advancement of organic chemistry towards the biological sciences and the technologies of medicine, it is becoming more and more difficult for any chemist to have an adequate grasp of the principles underlying many types of physical measurements that he may wish to carry out, or of potentialities of available apparatus. It is therefore necessary for one to be able to find easily, on library shelves, authoritative treatises that review, in an up-to-date manner, the technological potential of both classical and modern physics. Since 1946 the series of reference volumes, edited by Arnold Weissberger and issued by Interscience Publishers under the general title *Techniques of Organic Chemistry*, has been recognized as a comprehensive, reliable, publication of this character, though why these books should, by title, be directed more specifically to organic than to inorganic chemists appears to be but a fad of the publishers.

Books of reference must be kept up to date, and now Vol. 1 of the series, that is, *Physical Methods of Organic Chemistry*, has been divided into four parts. Two of these parts have appeared so far in 900-page volumes costing more than £9 each. The periodic reprinting and modernizing of a standard reference work is essential, but in this case the wisdom of the American motto 'bigger and better' can be questioned, for a number of the chapters of these new volumes strike me as being obsolescent in that they deal with measurements of types that few chemists and fewer physicists would deem worth conducting to-day. Still, the book has been revised thoroughly throughout.

Part 1 contains new, short, chapters on automatic control, automatic recording, weighing (mainly a summary of formal theory) and determination of particle size and weight. These are of importance to technologists who need to service complex instruments. Older chapters, often expanded, deal with temperature measurement, calorimetry, determinations of melting and boiling points, of vapour pressure, solubility, viscosity, surface tension, osmotic pressure and with work on mono-layers. Part 2 contains one new chapter. This deals with X-ray microscopy, which, as the writer admits, has not yet much applicability to chemistry. The others deal with diffusivity, the use of the ultracentrifuge, with refractometry, with crystal measurement, with microscopy (in great detail), with X-ray crystallography (far too briefly) and with both electron and neutron diffraction. Few of these have been changed significantly in this edition, and indeed there is little of new import to be said about several of these techniques. However, it is rational to survey established experimental methods before dealing with others of more recent origin. Furthermore it is to be hoped that the forthcoming Parts 3 and 4, which are to deal, *inter alia*, with spectroscopy and both nuclear and paramagnetic resonance, will show that the overall expansion of the treatise is fully justified.

Too much of these first two volumes has evidently been written by physicists for chemists, and a common attitude seems to have been "here is what can be done with this type of measurement ; why you should wish to use it is not my concern". So much is the chemical approach missing that I noted quite a few instances in which no mention had been made of novel, yet significant, applications of old techniques, as, for example, uses of calorimetry and of interference refractometry in studying rapid chemical reactions, or of solubility and vapour pressure measurements in determining the purity of optically active compounds.

Many users may find the chapters too theoretical for their liking, but in this respect they would do well to note the dictum of one contributor that "the experimenter should know more of his subject than he needs to apply in attaining the particular accuracy of his present measurements". This caution was rightly followed by notes on the theoretical and experimental limitations of commercial instruments. Such sections throughout the book are well worth reading.

Every specialist who consults this book may find gaps in the treatment of his own topic, but one should be circumspect in not demanding too much of any treatise.

W. A. WATERS

HUMAN GROWTH

Human Growth

Edited by J. M. Tanner. (Symposia of the Society for the Study of Human Biology, Vol. 3.) Pp. vii + 120. (London and New York: Pergamon Press, 1960.) 30*s*. net.

THIS, the third volume published on behalf of the Society for the Study of Human Biology, is a record of the proceedings of one of the symposia which the Society convenes periodically (see *Nature*, 186, 19 ; 1960). The topics are many and varied and range from age-changes in primates and their modification in man, contributed by Prof. Adolph Schultz of the University of Zurich, to the effects of race and climate on human growth as exemplified by studies on African children. The latter studies were initiated by D. F. Roberts, University of Oxford, and are of particular significance because the samples were taken not from dwellers in the vicinity of administrative and urban centres, but from remote peoples in tribal and rural areas. They corroborate previous findings showing that African children differ from European children by a tendency towards lower weight for a given height.

Two contributions to the symposium should commend this book to all educationists. The first, by Dr. Roy M. Acheson, examines the effects of nutrition and disease on human growth, and brings ample experimental evidence to show that even a very brief insult can greatly retard the growth of a child. The other, by Dr. D. W. Boyne, is concerned with secular changes in the stature of adults and the growth of children, especially relating to changes in the intelligence of 11-year olds. Besides confirming that the intelligence of boys and girls varies with respect to one another at different ages, the author produces additional evidence to confirm that the intelligence of girls over the age of eleven has been improving relative to that of boys since before 1939. Whether this is due to real change in the innate intelligence or to change in the nature of the tests is not known. It is certain, however, that great progress in our knowledge of human growth has been made since Sir D'Arcy Thompson concerned himself with biological gradients and that, besides educationists, this book has much to interest anatomists, zoologists, anthropologists and medical practitioners.

DECISIONS, VALUES AND GROUPS

Decisions, Values and Groups

Vol. 1 : Reports from the First Interdisciplinary Conference in the Behavioral Science Division, held at the University of New Mexico. Edited by Dorothy Willner. (Sponsored by the Air Force Office of Scientific Research.) Pp. xxix + 348. (London and New York : Pergamon Press, 1960.) 80*s*. net.

THE foreword of this book is by Dr. Charles E Hutchinson, chief of the Behavioral Science Division of the Air Force Office of Scientific Research in Washington; the introduction is by Dr. Anatol Rapoport; and the preface is by the editor. It is divided into five parts : (1) Mathematical Models in Decision Processes ; (2) Conceptualizations and Designs for Research in Values ; (3) Theoretical Contributions to Small Group Research ; (4) Psychodynamic Patterns of Behaviour ; (5) Special Military Problems.

There are twenty-three independent papers, too many even to list. Only one paper necessitates an acquaintance with higher mathematics. It is entitled "Stochastic Choice and Subjective Probability". But the experiment conducted would be regarded as somewhat simple by a student of Prof. M. S. Bartlett's *Stochastic Processes*.

The significant characteristics of the book seem to be that decision processes and evaluative processes are regarded as essential scientific concepts. That is, no science can proceed without a system of values. But here lie the most difficult problems of human endeavour and "no quick-fixes or break-throughs" are claimed. Perhaps progress will be reported in Vol. 2.

The method of attack was to select a large team of highly qualified younger scientists from various behavioural sciences. Each was assigned a problem and they all attended an eight-week work-session during June 17–August 10, 1957, for interdisciplinary activity, that is, a kind of prolonged super-symposium. Such a method clearly has advantages, but there is the view that new ideas are produced by individuals and not by committees, the young research worker receiving daily inspiration and guidance in the laboratory of his chief. Both methods may be combined.

Dr. Rapoport writes: "The title of this volume reflects a delineation of areas of study which is different from the traditional delineations suggested by university departments. There is no university department concerned with either 'decisions', 'values', or 'groups'. Decisions are studied, of course, by psychologists, by political scientists, and by military strategists. A 'decision theory', however, should transcend these special content areas". Similarly, "Values are studied by philosophers, by economists, by psychiatrists and by anthropologists from different points of view and by different methods".

The change of pattern in air-force research during the past forty-five years is obvious. In the First World War it was largely sensory. Many of the problems were solved under the direction of two men working in close collaboration, the physiologist, General Wilmer of the United States, and the psychologist, Prof. Spearman of Britain. Thus, when more than seventy ace pilots were tested by means of a battery of ocular tests, it was found that most tended to do well. One exception, who did badly in one test, was a famous ace who lacked the ability to land his plane undamaged. He was always given another. In the Second World War, air force research was more concerned with intellectual capacities. At the present time, we have noted that research has embarked on the scientific study of values. Is it too sanguine a hope that this will eventually help to abolish war?

Ll. Wynn Jones

DISEASES OF ORNAMENTAL PLANTS

Diseases and Pests of Ornamental Plants

By Pascal P. Pirone, Bernard O. Dodge and Harold W. Rickett. Third edition. (An official publication of the New York Botanical Garden.) Pp. x+775. (New York: The Ronald Press Company, 1960.) 10 dollars.

THIS book follows very closely the plan of the second edition but has been brought up to date by the inclusion of the latest information concerning the control of pests and diseases. In addition, the authors have revised the nomenclature of the pathogens to accord with the present views of taxonomists and plant pathologists. It is also evident from the increased number of entries under the various host plants that many new pests and diseases have been recorded in the United States since the publication of the previous edition twelve years ago.

The book is divided into two parts. The first contains an introductory chapter on "Plant Diseases" in which the diseases are classified and described under symptoms and causes. This is followed by a chapter entitled "Bacteria and Fungi" (viruses are, rather surprisingly, omitted) in which the structure and classification of these organisms are briefly discussed. It is here that one finds (p. 39) the rather sweeping statement that "Recent improvements in staining techniques, however, have revealed one to three septa (cross-walls), showing the bacterial body actually consists of two to four cells". Likewise the observation (p. 42) that "The threads (hyphæ) of many fungi are composed of fundamental structural units, cells, found generally in plants and animals" is apt to be misleading as it stands. Mycologists will also, no doubt, be surprised to learn (p. 66) that "It should be understood, that every species of fungus now classed among the imperfect fungi will ultimately be found to be connected either with some ascomycete, basidiomycete or phycomycete". The third chapter is devoted to a general account of "Insect and Other Animal Pests" and is followed by one on "Control". This latter chapter contains much useful information on such topics as disinfection of seeds, bulbs, tools, clothing and hands; pasteurization of soil; fungicides, bactericides, insecticides and miticides; spreaders; and equipment. General advice on the choice, preparation and application of fungicides, bactericides and pesticides is also included.

The second part of the book consists of an account of the individual pests and diseases of particular host plants, which are arranged in alphabetical order according to their Latin names. This section appears to cover the subject very thoroughly, although there are omissions such as the failure to mention witches' brooms of *Carpinus* caused by *Taphrina* spp., but such instances are few.

Detailed comparison between the entries of the fungus diseases in this work with those of the second edition shows a number of omissions, but these would appear to be due to the authors having restricted themselves to diseases occurring in the United States. Sometimes, however, the wording in the second edition is such that one is not always clear whether the disease actually occurred in that country. There is one rather odd instance of an entry in the second edition under *Anemone* of rhizome-rot said to be caused by *Sclerotinia tuberosa* for which symptoms are given together with a full account of the fungus. The disease is said to be "irregular in its occurrence. It is found naturally on wild plants, but is not common in gardens around New York City and in cultivated plantings; it can be very destructive", all of which clearly implies its presence in the United States. In the third edition under review there is also an entry of rhizome-rot under the same host, but here the causative agents are said to be *Pellicularia rolfsii* and *Sclerotium delphinii*. No mention is made of a disease caused by *Sclerotinia tuberosa*.

In conclusion, it may be stated that the appearance of this third edition of *Diseases and Pests of Ornamental Plants* will be welcomed by plant pathologists and growers, especially those in the United States, although its usefulness is by no means confined to that country. Its faults are few and relatively unimportant since they are unlikely to cause serious inconvenience to the average person using this volume as a work of reference. It is attractively produced and very well illustrated. Furthermore it is printed in larger type than its predecessor which makes for easier reading.

Derek A. Reid

STREAMING OF INTERSTELLAR HYDROGEN IN THE VICINITY OF THE SUN

By Dr. R. X. McGEE, Dr. J. D. MURRAY and Dr. J. L. PAWSEY, F.R.S.

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OBSERVATIONS of the 21-cm. hydrogen line have provided means for detecting the bulk movement of interstellar gas in our Galaxy. In addition to the well-known rotation about the galactic centre, Oort and Rougoor[1] have recently shown that the gas in the galactic disk, a few kiloparsecs from the centre, displays expansion at velocities of about 100 km./sec. It has been suggested by Kerr[2] that the outward movement may continue, with much reduced velocity, beyond the Sun. If this is so, where does the gas go? Does it stream outwards, above and below the disk? The results to be described here supply evidence for large-scale streaming of the gas in the vicinity of the Sun, but the observed flow does not conform with this simple picture.

The distribution of local neutral hydrogen has been discussed previously by Erickson, Helfer and Tatel[3] and by Davies[4]. We have recently completed a series of 21-cm. observations over the whole sky visible from Sydney with an aerial beam of 2·2° between half-power points and an *H*-line receiver of 48 channels spread at frequency intervals equivalent to 7 km./sec. in radial velocity about the natural line frequency. A full description of this equipment and the analysis of the observations is being prepared for publication.

One of the parameters extracted from the observations is $N_{\rm H}$, the number of hydrogen atoms in a line-of-sight column of 1 cm.^{2} section. It is derived from the integrated area of an *H*-line profile. Since the hydrogen is known to be concentrated in a thin layer (only a few hundred parsecs thick), most of the observations, except those near the Milky Way, will refer to gas in the vicinity of the Sun, that is, within a few layer thicknesses. A study of the distribution of $N_{\rm H}$ in galactic latitude showed it to be consistent with a general stratification parallel to the galactic plane in which a number of large clouds are embedded. These clouds include the Scorpius-Ophiuchus complex subtending some 3,000 square degrees, a large region in Gemini-Taurus-Orion, and its possible extension into Andromeda and Pisces. The examples mentioned are also characterized by *H*-line profiles the half-widths of which are less than 20 km./sec. Such evidence suggests that these local gas clouds are of a compact nature.

An approximate estimate of the neutral hydrogen density near the Sun has been made using: (1) average values of $N_{\rm H}$ at the different latitudes, and (2) the shape of the distribution in the z-direction (that is, perpendicular to the galactic plane) as derived by Schmidt[5]. The density is 0·46 hydrogen atom cm.$^{-3}$, which may be compared with 0·48 hydrogen atom cm.$^{-3}$, obtained by Westerhout[6], for the mean density between circles 8 and 9 kiloparsec. from the galactic centre. It would therefore appear that the Sun is situated in a representative region of the galactic disk.

Another parameter selected for study is the profile peak radial velocity. We have chosen the peak value since a great majority of the local profiles are single peaked and reasonably symmetrical. Thus the peak velocity gives a good representation of the gas velocity and, further, it may be easily and accurately read from the observed profiles. The velocities are referred to the local standard of rest.

The distribution of peak radial velocities is illustrated by the contour diagram of Fig. 1. The 'old' galactic co-ordinate system (Ohlsson) is used and, following convention, $l^{\rm I}$ and $b^{\rm I}$ designate galactic longitude and galactic latitude respectively. This is essentially a schematic diagram based originally on sample data analysis at intervals of 30° and 2° in $l^{\rm I}$ and $b^{\rm I}$, but afterwards checked at more frequent intervals. The contour interval is 3 km./sec. except for the zero velocity, shown in white, which includes -1, 0, $+1$ km./sec. values. Positive velocity regions are indicated by light grey and negative velocities by dark grey shading. In the Milky Way region an attempt was made to pick out only the contribution of the local gas, but uncertainties exist here due to possible inclusion of contributions from gas in other spiral arms. Radial velocities exceed 15 km./sec. in magnitude and reach maximum values of ~ 33 km./sec. in the hatched areas. Velocity values taken from the Leyden profiles[7] and Erickson *et al.*[3] have been used to augment the diagram in the region not visible from Sydney.

The striking feature of the velocity distribution is the extent of the areas of velocities of similar sign and magnitude. Two regions of positive velocity, one centred approximately on $l^{\rm I} = 10°$ and the other on $l^{\rm I} = 190°$, extend from the equator to galactic latitudes $\pm 40°$ over longitude ranges of more than 90° each. Regions of negative velocity lie between these at low latitudes. Negative velocities dominate the picture at high latitudes; the southern cap down to $b^{\rm I} = -40°$ is almost completely occupied by approaching gas, the northern cap has approaching gas together with a narrow sector near $l^{\rm I} = 150°$ of diffuse gas of various velocities including those of approach. The prevalence of negative velocities in the polar caps was noted by Erickson *et al.*[3].

The alternating positive and negative velocities in latitudes up to $\pm 30°$ are clearly associated with differential galactic rotation. It would be of great interest if, in addition, a general gas-flow pattern could be recognized. However, due to the lack of a measure of distance to the observed hydrogen, we cannot assign a particular velocity to be expected in a given direction due to differential galactic

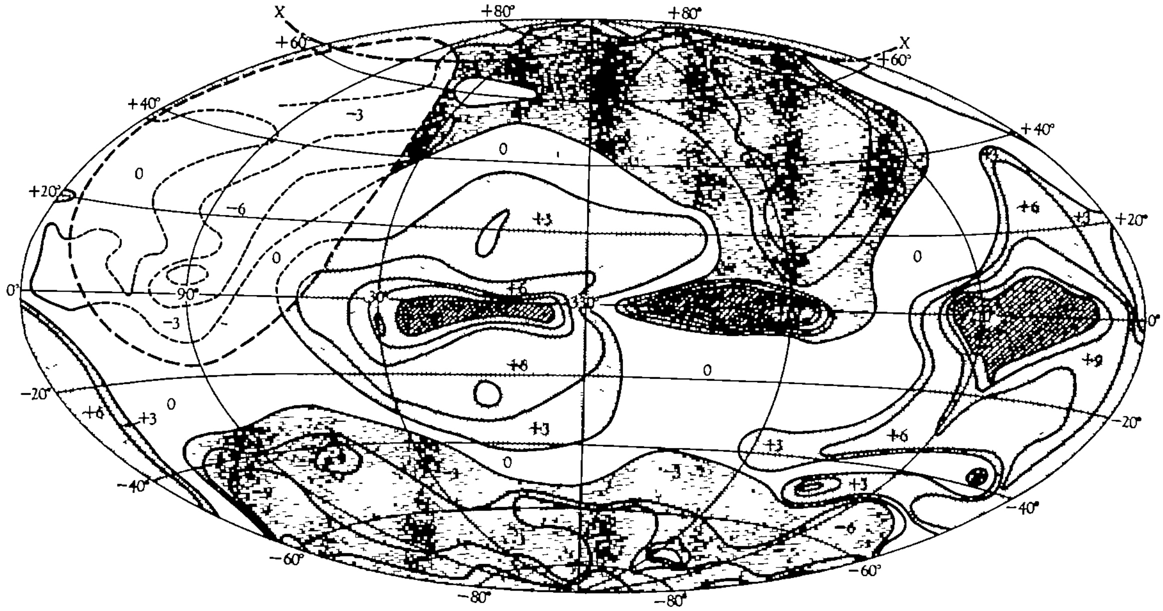

Fig. 1. The distribution of *H*-line peak radial velocities. The contour interval is 3 km./sec. Co-ordinates · 'Old' System, Galactic (Ohlsson). Light grey shading denotes regions of positive velocity, dark grey negative velocities, and white zero velocity (−1, 0, +1 km./sec.). Hatching denotes regions of velocity exceeding 15 km./sec. Diffuse gas of various peak velocities was observed in the region north of xx

rotation. But assuming the current rotational model and also that the Sun lies near the central plane of a stratified region of thickness $\leqslant 2z$ we can estimate an upper limit to the radial velocity, v_g, to be expected in a given direction (l^{I}, b^{I}), namely:

$$v_g \leqslant Az \cot b^{\mathrm{I}} \sin 2l^{\mathrm{II}} \cos b^{\mathrm{I}}$$

where A is Oort's constant (= 19·5 km./sec./k.parsec) and l^{II} the galactic longitude in the revised system in which the galactic centre is in the direction $l^{\mathrm{II}} = 0$ ($l^{\mathrm{II}} \approx l^{\mathrm{I}} - 327{\cdot}7$ at low latitudes). z has been taken as 0·11 k.parsec, Schmidt's estimate of the semi-thickness of the hydrogen layer to half density.

Examples of the derived curves are compared with the observed radial velocities in Fig. 2. Two quite definite results emerge. The first follows from the above equation in which the amplitude of the theoretical velocity curve falls to about 1 km./sec. above $|b^{\mathrm{I}}| = 45°$. In effect, then, the observed velocities in high latitudes are scarcely influenced by differential galactic rotation and hence local in-streaming of the gas is indicated.

The second result is due to the fact that, no matter how inaccurate the value of z may be, the radial velocity due to differential rotation in circular orbits must pass through zero at the longitudes of the galactic centre and anti-centre (327·7° and 147·7° respectively). The existence of observed positive velocities in these directions, and in directions where the model predicts negative velocities, emphasized in Fig. 2 by the thick segments, means that here the gas has an additional outwards motion. We consider that at low latitudes the other differences between observed and predicted values are within the uncertainty in interpretation.

Referring to Fig. 1 we conclude that at high latitudes, in the range $90° > |b^{\mathrm{I}}| > 40°$, neutral hydrogen is definitely flowing in from above and below at approximately 6 km./sec. at all longitudes. At low and middle latitudes the gas is flowing away

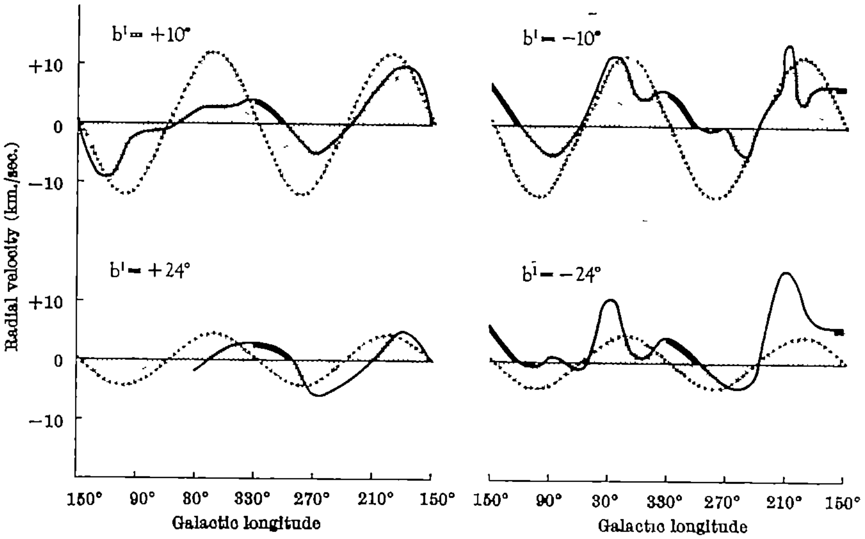

Fig. 2. Examples of curves of radial velocity against galactic longitude. The full curves show observed velocities. The shaded areas give the range of predicted velocities due to differential galactic rotation. The thickened segments of the observational curves indicate definite discrepancies between observation and these predictions

from the Sun in the general directions of the galactic centre and in southern latitudes near the anti-centre at approximately 6 km./sec.

We describe these motions as streaming, but we cannot tell if they are peculiar to our vicinity or whether they form part of a general circulation. Quantitatively, for the polar caps, combination of the velocity of inflow of 6 km./sec. with an estimated density of about 0·5 hydrogen atom cm.$^{-3}$ leads to a rate of inflow of about $10^{-7} M_{\odot}$ (solar mass) per square parsec per year. If this flow is general over a substantial part of the galactic disk, the amount of gas flowing in over a ring of 1 k.parsec width at the radius of the Sun would be several solar masses per year, so that the quantities of hydrogen involved would be sufficiently great to make the phenomenon a major feature of galactic dynamics.

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A HYPOTHESIS CONCERNING THE MECHANISM OF GENE ACTION

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Components of a Gene-System

It is usual to think of the gene as a particle located on the chromosome; but the phenomena associated with the action of a given gene occur in various places throughout the cell and should be considered to comprise a system which I shall designate the gene-system. The gene-system includes at least three separable entities: the receptor, located on the chromosome at the place usually spoken of as the locus of the gene, the transmittor and the effector. This discrimination is based on the fact that the gene is on the chromosome, and the enzyme which the gene controls is almost certainly synthesized in the cytoplasm, since the cytoplasm is the principal site of protein synthesis.

A Gene-System in Saccharomyces

The gene-controlled synthesis of an enzyme, such as melezitase[1,2], in the yeast cell, is initiated by exposure of the cell carrying the gene to various short-chain α-glucosides. In cells carrying the gene controlling the synthesis of melezitase, each of the different α-glucosidic inductors initiates the synthesis at the locus of the gene on the chromosome. The initiatory event results in the production of an enzyme which appears to have the same characteristics irrespective of the inductor. The mechanism which connects the induction at the locus with the final synthesis of enzyme in the cytoplasm is designated the transmittor. Melezitase (which is not present in an unadapted cell)[2] splits many relatively short-chained α-glucosides. It is induced by the five different α-glucosides, namely, turanose (*T*), maltose (*M*), sucrose (*S*), α-methyl-glucoside (*G*) and melezitose (*Z*). The letters *TMSGZ* are used to designate the dominant allele of the gene[3]. Unadapted cells require more than 1 hr. to accumulate detectable amounts of enzyme. The enzyme induced by any one of the inductors acts on all the other inductors and a few other short-chain α-glucosides[2,4] without an induction period, that is, without further adaptation. Because the different inductors seem to initiate the production of the same enzyme, it is inferred that both the transmittor and the effector respond to a stimulus initiated at the receptor, resulting in the production of a single uniform product, irrespective of which one of the five inductors acted as the inducing agent. Any inductor can activate the effector; the effector reacts non-specifically with respect to the specific inductor. These facts led to the hypothesis that the receptor[5], in the nucleus, which responds to a specified variety of inductors, and the effector, in the cytoplasm, which acts uniformly irrespective of which of the specified inductors initiates the action, must be connected by a transmittor.

Anatomy of the Receptor

Some ideas about the anatomy of the receptor have been suggested from the study of a series of multiple alleles which occupy the *TMSGZ* locus. The multiple alleles are distinguished from each other by their different abilities to respond to the five different inductors. Because there are five separable characters, thirty-two different possible multiple alleles might be expected if each character were expressed independently of the other four characters[3]. One allele, *TMSGZ*, responds to any one of the five inductors by the production of enzyme. At the other extreme is the totally recessive allele, designated by five dashes (– – – – –), which does not respond to any of the five inductors. Five other partially recessive alleles of the locus have been discovered: *TMSG–*, *TMS–Z*, *TMS– –*, *TM – – –*, *T– – – –*. Cells carrying these alleles respond to the inductors indicated by the capital letters but are incapable of responding to the substances indicated by the dashes. The limitation of the number of alleles proves that the characters are not expressed independently of each other. The fact that only one allele is incapable of responding to turanose as an inductor and that this allele is also incapable of responding to any other inductor indicates that the turanose character is fundamental to the receptor. This suggests that an injury to the locus which renders it incapable of responding to turanose will impair the capacity of the gene-system to respond to any other inductor. The limitation of the number of alleles suggested the hypothesis that the receptor is a conformation composed of at least five different overlapping patterns each composed of one or more elements[5,6]. Each of the patterns is assumed to fit one of the five inductors and to act as the specific receptor for that particular inductor. The elements of a pattern are assumed to form a template, or a reverse model, or a 'cast', of the inductor. Deformation of any element of a pattern may result in the inability of the particular inductor, for which the pattern is a receptor, to fit the inductor. The various patterns of the total conformation are assumed to overlap in such a manner that the turanose pattern is a component part of each of the other patterns, and it is assumed that injury to an element of the turanose pattern will make it impossible for any of the inductors to fit the conformation. But deformation of an element comprising the melezitose pattern will not prevent the other four inductors from fitting the conformation and bringing forth the production of enzyme.

Mutation by Ultra-violet Rays

Analysis of the mutagenic effects of ultra-violet rays on *TMSGZ* suggests that each pattern has a specifically different sensitivity to ultra-violet rays[3]. When *TMSGZ* is subjected to ultra-violet radiation the different alleles are recovered from among the survivors with approximately the following frequencies: *TMSG–*, 8 per cent; *TMS – –*, 4 per cent; *TM – – –*, 2 per cent; *T – – – –*, about 2 per cent; and – – – – –, less than 1 per cent. If one assumes that *TMSG –* results from injury to the *Z* pattern, the results indicate that the *Z* pattern is more sensitive than any other pattern. Likewise, if one assumes that – – – – – is the result of injury to the *T* pattern, the results indicate that the *T* pattern is more resistant than any other pattern. The *M* and *S* patterns are inferred to be about equally sensitive, but more sensitive than the *T* pattern. The *G* pattern is about half as sensitive as the *Z* pattern. The sensitivities of the different patterns may be ranked in the following ascending order: *T, M, S, G, Z.*

Gene-Conversion

Multiple alleles are also produced by gene-conversion. The multiple allele, *TMS–Z*, was recovered from cross-breeding experiments presumably as the result of gene-conversion[1]; it was not discovered among the survivors of ultra-violet irradiation. This suggests that the mutation of *TMSGZ* to *TMS-Z* (which involves injury to the *G* pattern without injury to the *Z* pattern) may occur in a heterozygote but is not commonly produced by ultra-violet radiation. A complete series of alleles indistinguishable from those produced by ultra-violet irradiation was recovered by tetrad analysis of heterozygotes. It was inferred that this series of alleles had been produced by gene-conversion[7].

X-ray Mutations

A third procedure for producing the different multiple alleles is X-irradiation. In contrast to ultra-violet-induced mutation, treatment with X-rays has produced only two types of mutants from the *TMSGZ* dominant allele; namely, *TM – – –* and – – – – –[4]. Although ultra-violet rays may destroy one pattern without affecting contingent patterns, the results suggest that the effect of X-rays may be 'spread' over several contingent patterns. For example, the *TM – – –* mutant might result from a 'hit' in either the *G* or the *Z* pattern which spreads to include the *S* pattern. The – – – – – mutant (which is characterized by damage to the *T* pattern) might be the result of a 'hit' which spreads to the *T* pattern from as far 'away' as the *S* pattern. Many lethal mutants were probably produced by X-rays but were not recovered. The mutants which survived and were recovered might have been produced by impacts of low energy. These inferences are consistent with the generally held view that ultra-violet rays are much less devastating in their mutagenic action than the X-rays.

Gene-Transformation by Substrate

Gene-transformation is defined as controlled mutation produced by a chemical reagent. In the preceding experiments, mutations from normal to defective were described[3]. Several reports from this Laboratory have concerned controlled reverse mutation from defective to normal by exposure of cells to specific carbohydrates[8,9]. Exposure of different partially recessive members of the *TMSGZ* series of multiple alleles to various specific substrates (especially to melezitose) has effected reverse mutations from various partial recessives (*TMSG –*, *TMS – –*, *TM – – –*, *T – – – –*) to *TMSGZ*. The partially recessive alleles are all stable when carried in culture on glucose. That is, when glucose is the only carbohydrate in the culture medium, and the cells are not exposed to any other sugar, the cultures can be carried indefinitely in sub-culture without encountering any alleles except those of the original culture. Because of our experience with the mutagenic effect of saccharides on yeast, cultures in this Laboratory are never returned to the stored 'stock' cultures after having been grown on any sugar except glucose. The stock cultures are all grown on a complete natural medium of peptone and yeast extract. Mutations at the *TMSGZ* locus occur when cultures carrying the 'partially' recessive alleles *TMSG –*, *TMS–Z*, *TMS – –*, *TM – – –* and *T – – – –* (excluding the totally recessive – – – – – allele) are planted densely on an agar plate containing melezitose. For example, if 10^6 *TMSG –* cells are spread on an agar plate containing melezitose, a small number, approximately 1×10^{-7} *TMSGZ* cells, are recovered. Thus exposure of the partially recessive *TMSG –* culture to melezitose transforms the *TMSG –* gene from a partially recessive to the *TMSGZ* dominant. The other partially recessive alleles also mutate to *TMSGZ* in the same circumstances. All the different partially recessive alleles (*TMSG –*, *TMS – Z*, *TMS – –*, *TM – – –*, *T – – – –*) when exposed to melezitose mutate to the same allele and only to this allele—the totally dominant *TMSGZ*[10].

I suggest that the receptor is a protein folded on the surface of the chromosome. The phenomenon of melezitose-induced reverse mutation suggests that, in the presence of melezitose, a defective element (which is inferred to be a 'false' fold in the receptor-protein) becomes folded into a functional pattern in the 'new' receptor protein, thus correcting the defect. It appears that, in the absence of melezitose, each receptor-protein folds in the same manner its 'parent' protein had originally folded and that the folding pattern of each of the alleles is hereditary.

The fact that melezitose is a chemical substance which induces a specific mutation specifies it as a transforming agent.

The 'Respreading' Experiment

The fact that reverse mutations from *TMSG* – to *TMSGZ* do not occur at a detectable rate on glucose supports the view that melezitose is the mutagenic agent. Confirmation of this view is derived from Newcombe's "respreading" experiment[11]: if a dense suspension of cells is planted on a melezitose-agar plate and the cells are respread four hours later (after about 2 divisions have occurred) the number of revertant colonies appearing on the respread plate is statistically equivalent to the number which appears on a control melezitose-agar plate on which the cells were not respread. If respreading is delayed for 10 hr. (after each colony contains many more cells) an increase in *TMSGZ* colonies is observed. The respreading experiment proves that the fermenter cells were not originally present among the glucose-grown cells planted on melezitose-agar. Hence, the reverse mutations were produced by contact with melezitose. (The failure to obtain an increase in the number of fermenter colonies if respreading is performed within 4 hr. is not due to the failure to break up clusters of cells. This was demonstrated by respreading mixtures of fermenter and non-fermenter cells at 4 hr. When plates are inoculated with artificial mixtures, an increase in the number of fermenters consistent with the number of divisions which has occurred is always observed, indicating that even the small clusters of cells break up easily[8].) These observations do not exclude the possibility that some undetectable reverse mutations may occur at a very low rate. For example, if the rate were 1×10^{-12}, it would be difficult to sample a sufficient number of cells to make the test. Replica plating experiments[3] showed that the 'spontaneous' frequency of back mutation of the partially recessive allele is less than 1×10^{-8}.

The fact that no change in the number of resistant colonies occurs in the first 4 hr., during which at least 2 divisions have occurred, indicates that the mutation does not usually occur in any of the four cells which are produced during the first 2 divisions. The mutations appear to be delayed until after several generations of contact with the mutagenic carbohydrate, suggesting that growth of the cell is essential to the occurrence of the mutation. Since it is inferred that the mutation is due to a change in the folding of a protein-receptor, these results suggest that the change can only occur during the formation of a 'new' receptor and that the 'old' receptor remains unchanged, even in the presence of melezitose.

The fact that melezitose has the capacity to direct the mutation of a defective into a normal gene means that melezitose is a transforming agent. This experiment shows that agents other than nucleic acids have the capacity to produce gene-transformations.

Synergistic Effect of X-rays and Melezitose on Gene Transformation

Cells carrying the totally recessive allele, designated by 5 dashes (– – – – –), do not respond to any of the five inductors by the production of the adaptive enzyme, melezitase, and are refractory to the mutagenic effect of substrate in the absence of auxiliary treatment. When the totally recessive allele is spread on an agar plate containing melezitose, revertant colonies are not recovered. If, however, the culture is treated with X-rays before spreading on melezitose-agar a few dominant *TMSGZ* colonies are recovered[5,10]. When the frequency of recovered colonies was plotted against the X-ray dose, it was found that the mutagenic effect was produced by a single 'hit'.

Size of an Element

The results were used as the basis for calculating the size of the 'target'[12] and the calculation indicated that the element had a molecular weight of from 15 to 60. The determination of target size at a level much below that of a single nucleotide indicates that this mutation is due to changes in items smaller than single nucleotides, and, hence, that the 'element' is not a single nucleotide.

Refractory (Stable) Nature of the *T* Pattern

The ultra-violet-ray experiments dealt with the induction of 'loss' mutations (*TMSGZ* mutated to – – – – –; the '*T*' pattern from '*T*' to '–'); conversely, the X-ray-melezitose experiments involved the 'gain' mutation from – – – – – to *TMSGZ*, presumably by remodelling of the '*T*' pattern from '–' to '*T*'. X-rays seem to alter the turanose pattern in some way which permits melezitose to induce the mutation from – – – – – to *TMSGZ*, in which the turanose pattern is changed from one which does not fit turanose to one which does. The X-ray experiments showed that the – – – – – allele was refractory to the transformation (– to *T*) by substrate alone and that a previous treatment with X-rays was required to produce the change. The turanose pattern is also more resistant to mutation by ultra-violet rays from *TMSGZ* to – – – – – (*T* to –) than any of the other patterns – –. The turanose pattern resists mutation in either direction.

The turanose pattern appears to be the basic pattern, and all the other patterns seem to have the turanose pattern as an essential component. If the ability to ferment turanose were the only criterion by which the genotypes of the locus were identified, this locus would appear to be extraordinarily stable and of extremely high penetrance. The fruitfulness of the present inquiry has arisen from the fact that many different means were available for identifying the different multiple alleles.

Characteristics of the Enzyme produced by *TMSGZ*

Cells containing melezitase act on the five inductors immediately at different rates. In general, the adapted cells act most rapidly on turanose, next most rapidly on maltose; the rate on sucrose is slightly lower. The rates on alpha-methyl-glucoside and melezitose are much lower than the rate on sucrose. Although individual determinations are subject to some error, the lowest rate appears to be that on melezitose. This is true whether whole cells or cell-free preparations are tested; the similarity between cell-free preparations and whole cells excludes the possibility that the differences in rates might be due to differences in the permeability of the membrane to the different saccharides.

The Permeability Barrier

Data from two sources revealed that the different inductors enter the cell at the same rate. (1) Data on oxygen absorption of a *TMSGZ* culture in solutions of the different sugars supported the view that the different rates of fermentation were not due to different rates of entry[4]. When curves, describing the rates of oxygen absorption of the cells undergoing adaptation to the sugars in different Warburg vessels, are extrapolated, they intersect on the time axis at the same point. Thus, the period elapsing from the time at which substrate is added to the time at which the cells respond is the same for the different substrates, indicating that the time required to pass through the permeability barrier is the same irrespective of the inductor. But the rate of fermentation of each substrate is characteristically different and this is inferred to be due to characteristic differences between the rates at which the same enzyme splits the different substrates. (2) Data from cell-free preparations (in which permeability barriers have been destroyed) confirmed the fact that the different rates after adaptation are due to the differences with which the induced enzyme acts on the different inductors.

A POSSIBLE MECHANISM OF GENE ACTION

Parallels between the Enzyme and the Receptor

It is generally held that the enzymes which act in the cytoplasm, and are produced in the cytoplasm. are proteins. Thus, the enzyme, melezitase, which is assumed to be a protein, acts with different rates on the different inductors, cleaving each inductor at a specific and characteristic rate. The rates may be arranged in the descending order: turanose, maltose, sucrose, alpha-methyl-glucoside and melezitose. There is a significant parallel between: (1) the activity of the enzyme toward the different substrates and (2) the sensitivity of the different patterns to ultra-violet rays. The correlation between these two different phenomena invites the hypothesis that both the receptor of the gene-system and the enzyme are folded proteins which mimic the topology of the surface of the carbohydrate inductors. The capacity of the single *TMSGZ* conformation to fit the five different inductors is inferred to be due to the relationships of the basic turanose pattern to the overlapping and partially superimposed patterns of the other four inductor molecules. The argument that the receptor surface is *not* deoxyribonucleic acid (DNA) is supported by the fact that nucleic acid is not needed to transform the defective surface into a functional surface since the trisaccharide, melezitose, can transform any of the impaired or damaged surfaces, presumably by acting as a model on which a new receptor surface is formed.

Relation of DNA to the Locus of the Gene

The hypothesis that the receptor is a folded protein does not rule out the essential involvement of nucleotides in the gene-system. It is proposed that the receptor 'fits' the chromosome by being engaged to the coded nucleotides, either RNA or DNA, which comprise the chromosome at the specific locus of the gene. This theory rejects the view that the chromosome is simply a double helix of DNA. It proposes that the chromosome is a many-stranded structure with both protein and RNA associated at the locus. It also proposes that if coding of the nucleotides has significance, some single strands of DNA must be free to react at the locus of the gene. On this theory, the specific variations of the outer surface of the receptor depend partially on the contact of the inner surface of the receptor with the single strands of coded nucleotides, and the integrity of the receptor depends on contact with these coded nucleotides. Minor deviations in the shape of the receptor which depend simply on folding of the protein may be independent of the nucleotide sequence and may be corrected by refolding the protein in the presence of the carbohydrate inductor. (These are the partially recessive mutations which can be induced to undergo mutation to normal by treatment with the inductor.) But if the nucleotides are rearranged, the surface of the conformation, and hence the patterns which fit the inductor, are disturbed. Reactions which affect the synthesis of purines or pyrimidines could produce gene mutations by altering the 'seat' on which the receptor is folded. The coded sequence of the nucleotides at the locus is essential to the maintenance of the conformation characteristic of the receptor surface because it would determine the type of folding which will occur, thus affecting the outer surface of the receptor. The theory is consistent with the view that changes in the nucleotide arrangement of the chromosome could lead to mutation.

On this hypothesis DNAase should destroy transforming principle, since it would remove the DNA 'seat' on which the protein is folded and maintained in the normal conformation. Re-arrangement of nucleotides in the chromosome would tend to change a normal gene into a defective gene.

The theory proposes that the receptor-protein has: (1) an outer (functional) surface and (2) an inner surface in contact with the nucleotides. The fact that the partially recessive alleles are transmitted unchanged from generation to generation suggests that irregularities in the folding of the receptor-protein, such as those, for example, which differentiate *TMSGZ* from *TMS – –*, are transmitted from generation to generation. This phenomenon suggests that hydrogen bondings within the folded protein are transmitted from generation to generation. It is proposed that this effect is produced by the folding of the 'new' protein produced at each 'new' generation on the lateral surface of the 'old' protein. In the presence of melezitose the receptor-protein occasionally folds on both the nucleotide surface and the melezitose surface and the irregularities in folding which produced the partially recessive alleles are 'corrected' and *TMSGZ* is produced.

Relation of Synapsis to Transformation

I have discussed elsewhere[13] the importance of DNA in homologous synapsis and proposed that the DNA component of the transforming principle ensures that the 'transforming agent' will be fitted into its proper position in the chromosome by combining with the homologous complementary single strands of DNA on the chromosome.

The Particulate Gene

This theory implies a return to the theory of individual particulate gene and rejects the idea that genes are continuous[14]. The coded nucleotide serves: (1) to hold the receptor-protein in place; (2) to form a base on which the protein can fold in such a way that a functional conformation will result; and (3) to ensure the precise homologous synapsis which will prevent non-reciprocal or unequal crossing-over. On this theory, the gene is defined as a 'linear section of the chromosome capable of accommodating one species of a variety of closely related (allelic) receptors'. Steffensen's[15] theory of crossing-over, which states that the chromosome is a continuum in which nucleic acid segments are held together by Ca bonds, and that crossing-over occurs only at these Ca bonds, is consistent with the theory.

Crossing-over versus Other Kinds of Recombination

Crossing-over is defined as exchange occurring between chromatids by breakage and reunion which is usually reciprocal and is assumed to occur at the Steffensen bonds. Other kinds of recombinations may be due to gene changes by: (a) substitution, deletion or chemical alteration of nucleotides; (b) gene-conversion, gain or loss or alteration of receptor-protein; or (c) transduction. A critical discrimination between crossing-over and recombination due to causes other than crossing-over can be made only if tetrad analysis is available. Most of the low-frequency recombinations which have been revealed by viral and microbial genetical analysis are almost certainly not due to crossing-over.

Function of the Nucleolus

On this theory one element of the transmittor of each gene-system is the moiety of RNA[16] (the template) which passes from the nucleus into the cytoplasm to effect the synthesis of enzyme in the cytoplasm. The nucleolus is a temporary organelle appearing in a restricted region of a chromosome during the interphase between nuclear divisions. It is reasonable to suppose that the nucleolus is an organelle in which RNA is processed during its passage into the cytoplasm. The nucleolus affords an isolated site in which RNA could be altered without interfering with other reactions, especially reactions that occur in the cytoplasm. Thus the nucleolus would be an important component of the transmittor, and all that is known concerning the function of RNA in the synthesis of protein is consistent with the theory in explaining the synthesis of enzyme.

Gene-Conversion and Delayed Mutation

This theory suggests that the mechanism of gene-conversion might be different from that of transformation. Conversion from defective to normal, or *vice versa*, might occur in the heterozygote without breakage of the DNA strand of the chromosome and without involving crossing-over. It is proposed that conversion could be effected by the transfer of receptor-protein from the locus on one chromosome to the corresponding locus of its homologous partner. The stability of non-reciprocal recombinants in yeast heterozygotes[17] and the photo-reversibility of non-reciprocal recombinants[18] offer evidence in support of this hypothesis. Transfer of receptor-protein between homologous loci could produce the non-reciprocal recombinations detectable by tetrad analysis which are characteristic of gene-conversion. The stability of the multiple alleles produced by gene-conversion at meiosis suggests that only a few receptors are present on the meiotic chromosome. But it would be reasonable to suppose that many receptors are present at each locus of the mitotic chromosome. This could explain delayed mutations, because if a large population of receptor-proteins at a locus were predominantly normal, with only a few defective, many multiplications might occur before a gene containing predominantly defective receptors were produced. Variations in the penetrance, expressivity or potency of genes (or high rates of mutability) might be due to variations in the population of receptor-proteins at a locus.

Significance of the Parallel between Enzyme and Receptor

The new phenomenon revealed by the study of the *TMSGZ* alleles is the parallel between the properties of the receptor on the chromosome and the properties of the proteinaceous enzyme in the cytoplasm. The *TMSGZ* gene-system is identified by five characteristics: the ability to hydrolyse the five sugars—turanose, maltose, sucrose, α-methyl-glucoside and melezitose. Treatment of the cells with ultra-violet rays shows that the different patterns of the receptor vary in sensitivity to ultra-violet rays: the most sensitive pattern is the melezitose pattern; the most resistant pattern is the turanose pattern. The different patterns can be arranged in descending order according to ultra-violet sensitivity: *T*, *M*, *S*, *G* and *Z*. The changes in the gene are transmitted according to the rules of Mendelian heredity, proving that the experiments on sensitivity to ultra-violet rays pertain to the effects on the receptor located on the chromosome. But the characteristics of the enzyme involve events in the cytoplasm and the enzyme exhibits certain characteristics which parallel those of the gene. The substrates can be arranged in the following descending order according to the activity with which the enzyme acts on them: turanose, maltose, sucrose, α-methyl-glucoside and melezitose. The phenomenonological parallel between ultra-violet sensitivity of the patterns on the chromosome and the activity of the cytoplasmic proteinaceous enzyme suggest the possibility that both the receptor and the enzyme are protein. If the receptor-protein on the chromosome and the enzyme-protein in the cytoplasm are similar, the synthesis of enzyme in the cytoplasm involves a replication of the structure of the receptor in the cytoplasm. But this process must involve many intermediary steps because the synthesis of enzyme occurs in the cytoplasm far from the locus of the gene, where the induction occurred, and the protein itself does not pass from the nucleus into the cytoplasm. The biological economy achieved by constructing both receptors and enzymes from amino-acids probably lies in the fact that a pool of twenty activated amino-acids can serve as a source of protein which can be folded into almost any conceivable shape.

Mechanism of Gene Action

Enzymes are supposed to split carbohydrates by possessing a surface which almost fits the carbohydrate. If a disaccharide such as turanose impinges upon the enzyme surface it is subjected to distortion because the 'fit' is not perfect. This leads eventually to splitting the disaccharide into its two hexose components. The present theory of protein synthesis proposes that proteins are synthesized in the cytoplasm by the alignment of a series of activated amino-acids on a ribonucleic acid template[18]. The ribonucleic acid template assembles the amino-acids into a protein which is the enzyme. The ribonucleic acid template is a crucial component of the transmittor of the gene-system.

I shall propose that the RNA template arises from the locus of the gene on the chromosome. On this hypothesis there are some exposed nucleotides of single-stranded DNA available at the locus which bind RNA and receptor-protein to the locus. Double helices of DNA may also be present to give the chromosome structural integrity, but it is single-stranded DNA which is associated with the RNA and the receptor. At each locus the structural DNA is held in its place in the linear continuum of the chromosome by a calcium bond at each end. The locus is also occupied by an RNA nucleotide, corresponding in length and in the distribution of similar or complementary nucleotides with those of the DNA chain. But the RNA chain is not bound into the long axis of the chromosome by calcium bonds. The DNA-RNA-protein-receptor complex becomes unstable when the inductor unites with the receptor-protein and the RNA-template is released from the complex. Because the RNA-template is not calcium-bonded it is free to leave the locus and travel to the nucleolus, where it is processed for its passage through the nuclear membrane and its action in the cytoplasm.

POSSIBLE ORIGIN OF MENDELIAN GENES FROM ENVIRONMENTALLY ACQUIRED CHARACTERS

Origin of Adaptive Enzyme-forming Systems

I have suggested that the genome 'stores' the capacity to produce adaptive enzymes. The preceding hypothesis concerning the mechanism of gene-action may form a basis for a hypothesis concerning the origin of gene-systems. On this hypothesis, eobionts, which resembled the cytoplasmic-like coacervates conceived by Oparin[19], grew on the accumulated organic material which had been produced by abiotic syntheses, using (at one time) simple monosaccharides as energy sources. As the available monosaccharides became exhausted, accumulation of non-utilizable substances, such as disaccharides, intruded into the eobiont. The presence of the disaccharides impinging on the proteins, being formed by the eobiont, led to the construction of imperfect mirror images of the disaccharides by the same processes which lead antigens to initiate the construction of antibodies in the presence of unfolded proteins[20]. The imperfect fit to the surface of the disaccharide might produce a state of stress in the disaccharide molecule, causing the molecule to split and making a new source of energy in the form of monosaccharides available to the eobiont. The released 'enzyme' could then repeat the process. The disturbance produced by the disaccharide would lead to a temporary modification of the eobiont. So long as the 'enzymes' persisted a 'lingering modification' like that suggested by Jollos[21] would persist. The enzymatic process might have a very low rate, but in the absence of other sources of energy the rate would not be important. For many millennia energy might have been provided from new sources by this adaptive mechanism. Eventually some of the protein may have folded simultaneously, both on a ribonucleic acid and a saccharide, and the RNA would form a template for synthesis and folding of more enzyme, thus giving the eobiont an advantage, especially because replication of the nucleic acid might take place according to the Watson–Crick[22] hypothesis. A mechanism for replication of the template would reduce the time of adaptation by a large factor. Perpetuation of the enzyme in the cytoplasm would be assured, and the survival value of the virus-like RNA-cytoplasmic-template would increase the chances of survival of the eobiont.

But the theory thus far does not mention a nuclear apparatus. I have proposed that the genome is a recent development[23,24]. Hence, it remains to suggest a mechanism by which the capacity of the cytoplast to produce enzyme might have become stored in the genome. If DNA were available in the eobiont, or in its vicinity, a pattern reflecting the pattern of the cytoplasmic RNA might be present in the DNA. (It is assumed that these events may have occurred long before the origin of nuclei or cells, when the eobiont was only a slimy plasm.) If the nucleotides in the DNA were so arranged that the RNA template of the enzyme could become attached to the DNA by hydrogen bonds, the enzyme, carried by the RNA to the DNA, would become the protein-receptor of the chromosomal gene. The DNA-continuum would provide a locus on which the RNA-enzyme complex could be held in place and on which the RNA-template could duplicate the 'receptor-enzyme'. If this triple complex were supplied with the necessary materials for its continued replication, and if it could be isolated from the rest of the plasm, a genome capable of bringing forth the production of enzyme in the cytoplasm on contact with appropriate substrates would be established in the eobiont and might be expected to be maintained even during periods when no substrate was available.

Relation of Acquired Characters to Genes

The purpose of this article is to suggest the possibility of relating environmentally acquired characters to Mendelian genes. A hypothesis is presented which suggests that capacities to produce enzymes, acquired by the cytoplasm, eventually may become Mendelian genes. Unless the first eobiont carried the total potential exhibited by the great variety of living things, both past and present, it seems reasonable *a priori* that acquired characters must have become genic characters. Since no other hypothesis relating environmental modifications (acquired characters) to genes has been proposed, this hypothesis must serve to fill a void in theoretical biology until it can be replaced by one more acceptable.

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OBITUARIES

Prof. Maud Menten

Maud Menten, experimental pathologist, died on July 20, 1960, at the age of eighty-one in Leamington, Ontario. She was born in Lambton County, Canada, in 1879 and received the B.A. degree at the University of Toronto in 1904, the bachelor of medicine degree in 1907 and her M.D. in 1911. In 1916 she obtained a Ph.D. degree in biochemistry at the University of Chicago, where she studied with A. P. Mathews. Between 1904 and 1905 she was a demonstrator in physiology in the laboratories of A. B. Macallum. During 1907–8 she was a Research Fellow at the Rockefeller Institute for Medical Research, and, together with Simon Flexner and J. W. Jobling, was author of the first monograph of the Institute, a work on radiobromide and cancer. During 1910–12 and 1913–14 she was a Research Fellow at Western Reserve University in the sphere of Dr. George Crile. In 1913 she worked with L. Michaelis in Berlin, and this collaboration resulted in the famous paper on chemical kinetics which appeared in 1913 in the *Biochemische Zeitschrift*. In 1915–16 she did research in cancer at the Barnard Skin and Cancer Hospital in St. Louis, Missouri. In 1916 she joined the School of Medicine of the University of Pittsburgh, Pennsylvania, where she remained until her retirement in 1950. In Pittsburgh she was pathologist to the Children's Hospital of Pittsburgh from 1926 until 1950, and was a full-time teacher in the Department of Pathology of the Medical School; she rose to become a full professor of pathology. On retirement, she continued to do research work on cancer at the Medical Research Institute of British Columbia, until ill-health forced complete cessation of scientific activity in 1955.

Maud Menten was an avid research worker all her life. In Pittsburgh, despite full schedules as a hospital pathologist and teacher of pathology, she continued to make noteworthy contributions to scientific literature.

She was a versatile scientist, as becomes a pathologist. She will be best remembered at present for four pieces of work. Her work on enzyme kinetics with Michaelis in 1913 resulted in the Michaelis–Menten constant. In 1924, together with Helen Manning, she discovered the hyperglycæmic effects of salmonella toxins. In 1944, together with Andersch and Wilson, she determined the sedimentation constants and electrophoretic mobilities of adult and fœtal carboxylhæmoglobin; this has been stated to be the first use of electrophoretic mobility to determine differences in human hæmoglobin, antedating Linus Pauling. In 1944, together with Junge and Green, she developed an azo-dye coupling reaction for the demonstration of alkaline phosphatase in the kidney. This piece of work was called enthusiastically "a stroke of genius" by A. G. Pearse in the first edition of his book on *Histochemistry* because it opened up a field of enzyme histochemistry. Other research papers encompassed work on potassium in cells (with A. B. Macallum), radiobromide and cancer (with Simon Flexner and J. W. Jobling), oxidases, vitamin C (with C. G. King and O. Bessey), streptococcal toxins, histochemistry of glycogen, and nucleic acids in bone marrow. Other papers were published in the fields of physiology, chemotherapy, hæmatology and pathology.

Dr. Menten was a member of numerous scientific societies. Aside from science, she was a student of languages, music and art. She was a fine painter, and several of her canvases were hung in art exhibitions.

Maud Menten was untiring in her efforts on behalf of sick children. She was an inspiring teacher who stimulated medical students, resident physicians and research associates to their best efforts. She will long be remembered by her associates for her keen mind, for a certain dignity of manner, for unobtrusive modesty, for her wit, and above all for her enthusiasm for research.

Aaron H. Stock
Anna-Mary Carpenter

Prof. G. Bonnier

Prof. Gert Bonnier died on January 11 in Stockholm, where he had held the chair of genetics from 1936 until 1958. Even after his retirement he continued his experimental work until a few days before his death. He belonged to the generation of geneticists who, under the influence of the Morgan school, started to analyse the mechanism of heredity by means of experiments with *Drosophila*. One of his earliest papers, published before he had obtained his doctorate, is a thorough and ingenious study of secondary non-disjunction, which may gain renewed importance now that non-disjunction in man has proved to be a source of profound pathological disturbances. Crossing-over in triploids and attached *X*-chromosomes, chromatid interference, gynandromorphism and mosaicism were among the problems that interested him most. In 1947, together with B. and M. Rasmuson, he published a paper on "Gene Divisibility", which at the time of its appearance was somewhat of an oddity, but which now fits beautifully into the modern concept of the structure of the gene. After the War, Bonnier became increasingly interested in the genetical effects of X-rays and published a series of papers on this subject, several of them in collaboration with K. G. Luning, his successor to the chair. Perhaps the most interesting result of these studies was the finding that X-rays produce more mutations in spermatozoa when they are exposed in the inseminated female than when the male is treated. During the past few years, Bonnier's interest in radiation genetics shifted from these more fundamental aspects to the problem of radiation damage to populations, and he studied the genetical effects of X-rays in model *Drosophila* populations of different breeding structures and under different selection pressures. All these investigations are characterized by carefully thought-out and often novel techniques, by large and painstakingly collected data, by thorough statistical analyses and by cautious and critical interpretations.

To geneticists in general, Bonnier is probably best known as a pioneer in applied genetics. In 1928, he became the first chief of the Animal Breeding Institute in Wiad; he held this position until the Wallenberg Fund in 1950 withdrew its financial support. There he introduced the use of cattle twins for systematic studies of the effects of environment and nutrition on identical genotypes; similar groups of twins have now been collected and are being studied at many research stations throughout the world. He was also one of the pioneers of the use of artificial insemination in cattle, and the centre organized at Wiad in 1936 was the first of its kind in Scandinavia.

As a person, Bonnier was modest and shy, full of kindness and tolerance, and with a quiet sense of humour; he was respected by all who came in contact with him and loved by many friends and pupils. His father had owned one of the largest publishing houses in Sweden, and the tradition of culture and gracious living in which he had grown up was continued in the life of his own family. To have been a visitor in his beautiful house and to have enjoyed the warm hospitality of him and his wife is an unforgettable memory. His death will be deplored not only as a loss to genetics; it will also be a deeply felt personal loss for many people in many countries.

CHARLOTTE AUERBACH

NEW FELLOWS OF THE ROYAL SOCIETY

AT a meeting of the Royal Society on March 16, the following were elected to fellowship of the Society:

PROF. J. BADDILEY, professor of organic chemistry in the University of Durham, King's College, Newcastle upon Tyne, distinguished for his researches in organic and biochemistry, particularly in the field of coenzymes, nucleotides, and bacterial cell-wall constituents.

PROF. H. E. M. BARLOW, dean of the Faculty of Engineering, and Pender professor of electrical engineering and director of laboratories in the University of London, University College, distinguished for his work on engineering aspects of microwaves, particularly wave guides and semiconductors.

PROF. M. S. BARTLETT, professor of statistics in the University of London, University College, distinguished for his contributions to the methods of mathematical statistics and their application to physical and biological science.

PROF. R. E. BILLINGHAM, professor of zoology in the University of Pennsylvania, Philadelphia, distinguished for his studies on the pigmentary system of mammals and on tissue transplantation.

PROF. C. C. BUTLER, professor of physics in the University of London, Imperial College of Science and Technology, distinguished for his researches on the elementary unstable particles in cosmic rays.

DR. J. CHATT, head of the Inorganic Chemistry Department, Akers Research Laboratories, Imperial Chemical Industries, Ltd., Welwyn, Hertfordshire, and group manager of the Heavy Organic Chemicals Division, distinguished for his work on the chemical and physical constitution of co-ordinated metallic compounds.

PROF. B. H. FLOWERS, Langworthy professor of physics in the University of Manchester, distinguished for his numerous contributions to theoretical nuclear physics, especially the shell model of the atomic nucleus.

DR. L. H. GRAY, director of the British Empire Cancer Campaign Research Unit in Radiobiology, Mount Vernon Hospital and Radium Institute, Northwood, Middlesex, distinguished for his contributions to the effects of radiations on cells and to radiotherapy.

DR. S. H. HAUGHTON, inter-African scientific correspondent for geology, Commission for Technical Co-operation in Africa, Pretoria, formerly director of the Geological Survey of South Africa, distinguished for his many contributions to South African geology, especially in the fields of palæozoology, stratigraphy and economic geology.

PROF. RODNEY HILL, professor of applied mathematics in the University of Nottingham, distinguished for his contributions to the theory of continuous mediums, particularly to the plasticity of metals.

DR. H. E. HINTON, reader in entomology in the University of Bristol, distinguished for his work on insect biology, particularly on the larval stages of insects, and on classification and morphology of Coleoptera, Lepidoptera and Diptera.

DR. R. G. S. HUDSON, Research Fellow in the Department of Geology, Trinity College, Dublin, distinguished for his stratigraphical and palæontological researches in the Carboniferous of the North of England and in the Mesozoic rocks of the Middle East.

MR. C. F. KEARTON, managing director, Courtaulds, Ltd., Coventry, distinguished for his work on chemical engineering characteristics of volatile uranium products and for technological development in the textile industry.

Dr. H. London, deputy chief scientific officer at the Atomic Energy Research Establishment, Harwell, distinguished for his work on superconductivity, on liquid helium, and on the separation of isotopes.

Prof. M. Maizels, professor of clinical pathology in the University of London, University College Hospital Medical School, distinguished for his investigations into the mechanisms by which the electrolyte composition of animal cells is maintained.

Prof. Irene Manton, professor of botany in the University of Leeds, distinguished for her studies on the ultramicroscopic structure of plants and for studies on their evolution.

Dr. L. Marion, senior director, National Research Council of Canada, and director, Division of Pure Chemistry, Ottawa, distinguished for his work in organic chemistry, particularly in the field of alkaloids.

Prof. A. A. Miles, director of the Lister Institute of Preventive Medicine, London, and professor of experimental pathology in the University of London, distinguished for his contributions to bacteriology, and for his work on the mechanism of inflammatory reactions.

Prof. D. G. Northcott, Town Trust professor of pure mathematics in the University of Sheffield, distinguished for his work in abstract algebra and its application to algebraic geometry.

Dr. G. J. Popjak, director of the Medical Research Council Experimental Radiopathology Research Unit, Hammersmith Hospital, London, distinguished for his work on lipid metabolism and for elucidating the biosynthesis of fatty acids and cholesterol.

Dr. J. A. Pople, superintendent of the Basic Physics Division of the National Physical Laboratory, Teddington, Middlesex, distinguished for his work on the application of quantum mechanics to the explanation of the chemical and spectroscopic properties of molecules.

Dr. R. N. Robertson, executive of the Commonwealth Scientific and Industrial Research Organization, Melbourne, and professor designate of botany, University of Adelaide, distinguished for his contributions to plant physiology, particularly on salt accumulation and respiration and the ripening of fruits.

Dr. S. Siddiqui, director of the Pakistan Council of Scientific and Industrial Research, Karachi, distinguished for his work in the chemistry of natural products and for his leadership in the promotion of research in Pakistan.

Prof. A. W. Skempton, professor of civil engineering in the University of London, Imperial College of Science and Technology, distinguished for his pioneering work on soil mechanics, particularly in relation to the shear strength and other physical properties of clays.

Prof. M. J. D. White, professor of zoology in the University of Melbourne, distinguished for his contributions to animal cytology, particularly in relation to evolutionary processes.

NEWS and VIEWS

International Control of Investigations of Rare Meteorites

In recent years the study of meteorites has rapidly increased and, in the hands of investigators such as Urey, Anders, Wiik, Vinogradov and others, has thrown much light on the composition and origin of the solar system and of the planets. Last year, through the work of Mason on hydrous silicates in meteorites, interest has concentrated on the rarest form of meteorites, those which contain carbonaceous material. It follows from Mason's results, as well as from the earlier analyses of carbonaceous material by G. Mueller, that carbonaceous meteorites, unlike all other types, had never been subjected to high temperatures since their formation and that they might well contain samples of the raw materials from which life may have been formed on larger planets. On March 16, this hypothesis was given a new turn by the announcement at the meeting of the New York Academy of Sciences that Prof. B. Nagy, Dr. W. Meinschein and Dr. D. Hennessy had, by using mass spectroscopic and X-ray methods, found in the carbonaceous material from the meteorite which fell near Orgeuil in France on May 14, 1864, paraffinoid hydrocarbons characteristic of living organisms and hence had, in the opinions of these investigators, provided proof for the existence of life on its presumed parent body. This view is unlikely to go unchallenged, but whatever the final consensus of opinion, the crucial importance of this study of the carbonaceous meteorites is now evident.

The great publicity deservedly given to these observations and deductions has focused the attention of scientists in many countries on the carbonaceous meteorites. Paradoxically, the result may well be, unless appropriate steps are taken in time, to bring the researches to a rapid end by destroying the irreplaceable material on which they are based. There is known to be only some 30 kgm. of well-attested carbonaceous meteorites in museums. More, but not much more, may be in the hands of dealers or may be brought to light in other ways. Already these rare bodies are being examined in a dozen or more laboratories, each with a different objective and by methods which render the material useless for further study. This can only result in the exhaustion of the stock unless prompt measures are taken to ensure intranational and international co-operation similar to that in the various sections of the International Geophysical Year in the analysis of meteorites by different but complementary methods. The appropriate body for this is clearly the Committee on Space Research of the International Council of Scientific Unions, which, by a slight extension of its terms of reference, might cover bodies coming in from outer space as well as those going out into it. It is hoped that at the forthcoming meeting of the Committee at Florence, the matter can be discussed and appropriate measures taken in time to secure that the maximum of information is obtained from meteorites with the minimum of destruction.

Mathematics at Hull: Prof. G. C. Steward

THIS year Prof. G. C. Steward retires from the chair of mathematics in the University of Hull. He has occupied this chair since 1930, soon after the University College was first established. On his retirement, separate chairs of pure and applied mathematics are being instituted. Prof. Steward had his degree studies interrupted by the First World War, and he did not finally graduate at Cambridge until 1920. He was joint winner of a Smith's Prize in 1922. Meanwhile, he spent three years as assistant lecturer at Leeds before returning in 1923 to Cambridge as fellow and mathematical lecturer at Emmanuel College. He went directly from Cambridge to Hull. In 1918 he worked in the optics division of the National Physical Laboratory. This determined the course of his research work, which has been mainly on optical systems, using the eikonal function of Bruns, and other methods. He is the author of a Cambridge tract on symmetric optical instruments. In recent years he has been increasingly involved in departmental organization.

Prof. W. H. Cockcroft

DR. W. H. COCKCROFT, who is to be the first holder of the chair of pure mathematics in the University of Hull, was one of Prof. J. H. C. Whitehead's research students in the immediate post-war period. Since his doctorate at Oxford he has taught at the Universities of Aberdeen, Chicago and Southampton, where he is now a reader. His scientific interests are naturally directly derived from his early work under Whitehead. He was concerned in the first instance with various algebraic questions about group extensions and with some special problems arising in homotopy theory. He has since made contributions to homological algebra and topology generally. To mention one or two examples of research work of his, one may refer to some work which he and R. G. Swann have done in making improvements in the algebraic machinery used in Whitehead's work on simple homotopy types. In his work on the cohomology groups of a fibre space he has analysed the methods of the Belgian topologist Hirsch. In particular, he has obtained an explicit form for Hirsch's coboundary operator. This enables Cockcroft to make improvements in his own earlier work as well as to explain some results of Eilenberg–Maclane. Cockcroft has also taken great interest in the teaching of mathematics to undergraduates, and has taken part in symposia throughout the United States on the invitation of the National Science Foundation.

Prof. N. B. Slater

DR. N. B. SLATER, who has been appointed to the newly created chair of applied mathematics in the University of Hull, studied at the Universities of Edinburgh and Cambridge. For the year 1939–40 he was an observer at the Solar Physics Observatory, Cambridge; for the rest of the war years he was a member of Prof. L. Rosenhead's team in the Projectile Development Establishment. Since 1945 he has been on the applied mathematics staff of the University of Leeds, successively as lecturer, senior lecturer and reader. His Cambridge appointment led to a strong interest in amateur astronomy. It also led to an acquaintance with Sir Arthur Eddington, and, after the latter's death, Slater was asked to go through his unpublished manuscripts. He found that these cast a vivid light on the development of the ideas in Eddington's *Fundamental Theory*, and described what he found in a book published in 1957. However, Slater's main research interest has been in the theory of unimolecular gas reactions, on which he first began work under R. H. Fowler before 1939. In this field he has produced a standard theory, which has attracted a good deal of attention. A course given while visiting Cornell University was expanded by him into a book on the subject, which was published in 1959.

The National Reference Library for Science and Technology

IN an adjournment debate in the House of Commons on March 14, Mr. A. Albu asked a number of questions regarding the National Reference Library for Science and Technology. Stressing the value to the research worker of the open access system in the Patent Office Library, he questioned the adequacy of the proposed book stock of 500,000 current volumes, and thought that comparison with similar libraries elsewhere suggested a stock twenty times that proposed. He asked for specific assurances that the non-current volumes to be transferred to the British Museum would be readily available, that the site provided adequate room for expansion, and that measures were being taken to recruit staff of appropriate high scientific calibre. The Parliamentary Secretary for Science, Mr. D. Freeth, in his reply, said that it is not anticipated that there will be any overlapping or confusion of responsibility, although the Library will be part of the British Museum Library and come under the Chancellor of the Exchequer and not the Department of Scientific and Industrial Research and the Minister for Science. It will occupy a site to the east of the southern approach just north of the existing Cornwall Hall, and it is considered that the Library's total area of about 130,000 sq. ft. is well suited to house the 500,000 volumes required to provide the complete reference desired, together with an expert staff and open access. Reading rooms totalling about 22,000 sq. ft. will seat about 300 with seating room for a further 300 among the book stacks. The total cost is estimated at about £2·5 million.

Mr. Freeth said that the site does not provide room for all the expansion that might be desirable in future, but it is believed that it will provide a worthy national reference library, and that it will be possible to provide a speedy and adequate system for research workers to procure earlier publications from the British Museum. Legislation will be necessary for this purpose. The present stock of the Patent Office Library amounts to 210,000 volumes of periodicals, 100,000 volumes of patent specifications and 70,000 text-books; more than 7,500 current periodicals, including 300 Russian, are taken. Expenditure in 1960 was just under £180,000 and the staff numbered 72. Building plans have already reached an advanced stage, and Mr. Freeth said that any suggestions which prospective users might have for this building should be sent to the Principal Keeper in the Department of Printed Books in the British Museum, London, W.C.1, or to the Librarian, Patent Office Library, 25 Southampton Buildings, London, W.C.2. The exact constitution of the advisory committee to be set up when the new Library comes into operation is not yet decided. The Government is determined to see that the Library has an adequate expert staff ; there will be ample provision for photocopying, and the new Library will co-operate fully in research into information techniques.

Nestor: a New Reactor at Winfrith

A NEW 10-kW. reactor, *Nestor*, was put into operation for the first time at the Atomic Energy Establishment, Winfrith, on March 13. Its purpose is to act as a source of neutrons for sub-critical assemblies of nuclear fuels and moderators. The reactor core, control gear and instrumentation were designed and installed by the Hawker Siddeley Nuclear Power Co., which modified the design of its *Jason* reactor to enable up to five sub-critical assemblies to be driven simultaneously by the reactor. This concept represents a major advance in the use of a reactor as a neutron source. The Turriff Construction Corporation, Ltd., was responsible for the installation of the special shielding and the civil work generally. The nuclear fuel in *Nestor* is an alloy of highly enriched uranium and aluminium in the form of aluminium clad plates arranged in an annulus around a graphite cylinder, and natural water is used both as coolant and moderator. The reactor is provided with control and safety rods, and additional control is possible by varying the water-level. The experimental assemblies will be placed in 'caves' formed in the shielding at the sides and above the reactor. The whole shielding is arranged to be demountable so that the caves can be rebuilt to accommodate major changes in the requirements of the experimental programme.

The reactor is capable of operation at 10 kW. (heat), giving a thermal neutron flux of 10^{11} neutrons/cm.^{2}/sec. in the centre of the core and of up to 10^8 neutrons/cm.^{2}/sec. in the sub-assemblies. The thermal neutron flux for each sub-critical assembly can be varied by altering the reactor power-level, by using neutron shutters or by rearranging the fuel loading in the annular core.

Netherlands Atom Forum

A NUMBER of Netherlands industries together with the Reactor Centre of the Netherlands have taken the initiative for the establishment of the 'Netherlands Atom Forum'. The object of the new body is to give publicity to the developments in the field of nuclear energy, in particular to the activities in this domain, of the Netherlands industry and of institutes engaged in the subject. The intention is to do this by means of publications, the organization of conferences and information meetings, and the co-ordination of Netherlands participation in exhibitions. The Forum will also attend to the documentation and information on the development and application of nuclear energy, if possible in co-operation with existing organizations at home and abroad. It will in future represent the Netherlands in the 'Forum Atomique Européenne', which was established in Paris on July 12, 1960, and in which corresponding organizations from other Euratom countries, as well as from non-Euratom countries, have been brought together. Mr. W. A. de Haas, secretary of the Industrial Commission of the Reactor Centrum Nederland, has been elected chairman of the Netherlands Atom Forum ; the secretariat is accommodated at Reactor Centrum Nederland, Scheveningseweg 112, Den Haag.

Sea-water Purification Unit based on New Principles

A NEW type of evaporator developed for sea-water by the General Electric Co. of America may have application to other forms of heat exchangers, particularly because of the greater heat transference it achieves per unit area. The basis of its present form is a conventional steam-jacketed vertical copper tube 50 in. long and 6 in. diameter, from which evaporated vapour leaves at the top and concentrated brine at the bottom. The novel feature is the addition of wiper blades on a hollow shaft which sweep the inner surface of the tube at a speed of 300 ft./min. and spread sea-water issuing from the shaft and a header as a film some 0·001 in. thick on the wall. The tube is 0·05 in. thick and fluted lengthwise externally to provide increased heat exchange surface and improved removal of condensate; together with the wiper action heat transfer-rates of 40,000 B.TH.U./sq. ft./hr. can be obtained without perceptible carry-over of spray. It is claimed that with a throughput evaporation of 40 per cent a purity of one part of salt per million is achieved. The makers expect to realize a saving in weight and space of some 40 and 60 per cent, respectively, over conventional plants and have in mind systems evaporating 1,000–1,000,000 gallons daily.

Arts Graduates and Imperial Chemical Industries, Ltd.

A NOVEL method of directing attention to the opportunities existing at Imperial Chemical Industries, Ltd., for arts graduates is set out in an attractive handbook (*A Degree nearer Industry.* Pp. 40. By Kenneth Harris (London : Recruitment Section, Imperial Chemical Industries, Ltd.), 1960). Kenneth Harris, a well-known industrial journalist, was invited to carry out a survey of his own devising to find out what had happened to arts graduates in the different companies of the Imperial Chemical Industries, Ltd., during recent years. The report prepared by Harris is frank and not always complimentary to Imperial Chemical Industries, Ltd., but clearly shows that here is a company where young men of the right qualities are needed and can find their way to the top. The booklet could well be read by all arts graduates contemplating a career in industry—the picture painted by Harris is not dissimilar from that of many other large industrial organizations.

Durban Museum and Art Gallery

THE report of the Durban Museum and Art Gallery for the municipal year 1959–60 records the development of the new Marine Gallery on modern museum lines, and pleads once again for new buildings for both the Museum and Art Gallery (Pp. 20. Durban : Durban Museum and Art Gallery, 1960). A large and varied selection of temporary exhibitions were staged during the year and the collections at the Old House have been expanded and their display considerably improved.

British Technology Index

IF sufficient support is forthcoming, a new endeavour is to be made to index the contents of British technical periodicals. There have been at least two earlier attempts to do this, and it is probable that part of the reason for their failures was the absence of a cumulative index or volume. The new *Index* will have 11 monthly parts (no August issue) and a bound annual cumulation. It will be sponsored by the Library Association and will index about 400 journals, many of which are not already abstracted or indexed. It will produce each issue within six weeks of the publication of the journals concerned. The price will be about 15 guineas. Further particulars, and a prospectus containing a specimen page, are obtainable from the Association at Chaucer House, Malet Place, London, W.C.1.

Field Studies Council

That the Field Studies Council is performing a real service to learning is again clearly brought out in its annual report for the year 1959–60 (Pp. 32+4 plates. (London: Field Studies Council, 9 Devereux Court, W.C.2.) 1961). Between 1951 and 1960 the number of visitors to the centres has more than doubled and, in 1960, was well over 8,000. It is interesting that more than half the visitors went to study 'biological subjects', and more than 3,000 studied 'geographical and geological subjects'; only 315 students were concerned with the 'countryside and natural history subjects'. The figures reflect mounting pressure from grammar schools and training colleges, while the report indicates that the centres could have been filled many times over between March and Whitsuntide. During the year amenities at Slapton Ley were improved and the new geography block was completed at Dale Fort.

The World Meteorological Organization: 1961 Events

The World Meteorological Organization has announced its schedule of conferences and symposia to be organized during the rest of 1961: April 12–26, Commission for Hydrological Meteorology—first session (Washington); June–July, Regional Association III—South America—third session (Rio de Janeiro); August 6–12, international symposium on "Atmospheric Ozone and General Circulation", to be held in conjunction with the International Ozone Commission (Arosa); August 14–19, symposium on "Radiation", to be held in conjunction with the Radiation Commission of the International Association of Meteorology and Atmospheric Physics (Vienna); September 18–October 2, Commission for Aerology—third session (Rome); October 2–7, symposium on "Climatic Changes", to be held in conjunction with Unesco (Rome). Further information can be obtained from the Secretariat, World Meteorological Organization, Geneva.

The Night Sky in April

Full moon occurs on April 1d. 05h. 48m. U.T., new moon on April 15d. 05h. 38m. and full moon on April 30d. 18h. 41m. The following conjunctions with the Moon take place: April 9d. 06h., Saturn 3° S.; April 9d. 14h., Jupiter 3° S.; April 18d. 13h., Aldebaran 0·4° S.; April 22d. 05h., Mars 5° N.; April 25d. 05h., Regulus 1° N. In addition to these conjunctions with the Moon, Mercury is in conjunction with Venus on April 18d. 01h., Mercury being 9° S., and Mars with Pollux on April 23d. 03h., Mars being 5° S. Mercury is too close to the Sun for easy observation. Venus will be visible for a few days at the beginning of the month as a bright star low in the west after sunset, but later in the month it is too close to the Sun for observation. Mars sets at 2h. 50m., 2h. 20m. and 1h. 40m. on April 1, 15 and 30, respectively. Mars is an evening star, but conditions for observation are becoming less favourable. Its stellar magnitude on April 15 is + 1·1; its distance is rapidly increasing and its apparent diameter decreasing. Jupiter and Saturn are morning stars, both rising just over two hours before the Sun; conditions for observation are not favourable. There is one occultation of a star brighter than magnitude 6 during the month, observations being made at Greenwich: Aldebaran, disappearance April 18d. 12h. 24·3m., reappearance April 18d. 13h. 01·4m. The Lyrid meteors are active during April 20–22, the maximum being on April 22. The radiant is at R.A. 18h. 04m., Dec. + 33°, and conditions for observation are very favourable.

Announcements

Prof. Dragoslav Popovic has been appointed director of the International Atomic Energy Agency's Safeguards Division, in succession to Mr. R. M. Smith (Canada). Prof. Popovic is at present at the Boris Kidric Institute of Nuclear Science at Vinca, near Belgrade, and has been professor of nuclear reactor engineering at the University of Belgrade since 1958.

Dr. Karl F. Meyer, director emeritus and professor emeritus of the University of California's George Williams Hooper Foundation for Medical Research, has been awarded the Jessie Stevenson Kovalenko Medal of the U.S. National Academy of Sciences. The award has been made in recognition of Dr. Meyer's achievements as an investigator, teacher and administrator in medicine for more than half a century, and especially for his work on the role of animals as hosts for vectors of human disease.

The Worcester Foundation for Experimental Biology, Shrewsbury, Mass., with the aid of a grant from the Population Council, is continuing its post-doctoral programme for scientific training in reproductive physiology. Fellowships will be awarded to candidates possessing the Ph.D. or M.D. degrees, or their equivalents; they will carry a stipend of 5,500 dollars per annum and will be for a twelve-month period. Application forms can be obtained from Dr. Celso-Ramon Garcia, at the Worcester Foundation, and should be returned not later than June 1.

The British Hydromechanics Research Association is organizing an international meeting on "Fluid Sealing" at Grosvenor Hall, Ashford, Kent, during April 17–19. Further information can be obtained from Mr. H. Stephens, information officer, British Hydromechanics Research Association, South Road, Temple Fields, Harlow, Essex.

A symposium on "The Water Relations of Plants" is being organized by the British Ecological Society, and will be held at the Imperial College of Science and Technology, London, during April 5–8. Further information can be obtained from Dr. F. H. Whitehead, Botany Department, Imperial College of Science and Technology, Prince Consort Road, London, S.W.7.

The ninth annual general meeting and conference of the British Agricultural History Society is to be held at Seale Hayne Agricultural College, Newton Abbot, Devon, from the evening of April 5 until lunch-time April 7. Further information can be obtained from Mr. S. T. Morris, 1 Courtenay Park, Newton Abbot, Devon.

HISTORY AND PHILOSOPHY OF SCIENCE IN THE COMMONWEALTH

By Dr. W. MAYS

Department of Philosophy, University of Manchester

The General Problem

THE history and philosophy of science is a subject of increasing importance, both in the universities and outside them. In the universities, where the division between the sciences and the humanities is growing more acute, it has a most valuable part to play. By integrating diverse fields of study, it can do a great deal to ameliorate the evils of over-specialization.

Because of this it seemed worth while finding out how the subject was developing in the British Commonwealth universities, both in its teaching and research aspects. These universities form a fairly coherent unit, since, in most cases, they have been founded on the model of the British universities. To obtain this information, a questionnaire was sent out to a hundred universities, most of which were in Australia, Canada, India and South Africa. Out of these, thirty-nine reported activity in the history and philosophy of science, and to this information was added returns from twenty-one British universities[1].

Six groups of questions were asked: (1) was the university teaching the history and philosophy of science as a regular and independent part of the curriculum; (2) whether there was an established post or the expectation of one; (3) the type of course given; (4) the manner of treatment; (5) the value of the subject; (6) general remarks.

Value and Future of the Subject

Most of the replies stressed the value of the subject in university curricula. In the case of science students such courses were seen as a means of integrating knowledge of the physical world with the humanities and as a counter to specialization. So far as arts students were concerned, it was thought that courses of this sort enabled them to obtain a general scientific outlook, and made them conversant with the influence of science on philosophy and culture.

In most Commonwealth universities very few independent teaching appointments have as yet been made. Most of the lecturing appears to be of a stop-gap variety carried out by members of science, philosophy or education departments. What clearly emerged was that the most effective way of teaching the subject was through an autonomous department. In a modern university unless a subject has departmental independence and representation on the governing academic body, its chances of internal growth may be slight. Melbourne, the only Commonwealth university outside Britain to have an autonomous department, was firmly convinced of the benefits accruing from its independent position. It is also clear that in view of its importance, both as a subject in its own right and as an integrating discipline, there is a very strong case for the establishment of chairs in the history and philosophy of science, at least in the larger universities.

The following points were also made. More university teachers in the subject were needed: (a) for research, and (b) for undergraduate teaching. There was a need to include the history and philosophy of science as part of the general education of sixth-form pupils as well as undergraduates. As the demand for the subject in schools will probably expand, it would be useful to have courses in education departments for prospective teachers of the subject. Further, there would seem to be important teaching possibilities in the field of extra-mural studies.

Results of the Survey

In Great Britain the Universities of London, Oxford and Cambridge have well-established teaching and research departments. The oldest department is that of the History and Philosophy of Science at University College, London, founded nearly thirty years ago. It is, however, mainly concerned with postgraduate studies. Another London department is that of Philosophy, Logic, and Scientific Method at the London School of Economics, where both undergraduate and graduate courses are given. The Oxford and Cambridge Departments are more recent and are post-Second World War creations. At Cambridge, the history and philosophy of science may be taken as an optional half-subject in the Natural Sciences Tripos, and a certificate in the subject is also awarded. Oxford provides an extensive range of courses and awards a diploma. Research degrees may be taken at London, Oxford and Cambridge.

Aberdeen has a reader in the history and philosophy of science. In some modern universities a number of lectureships as well as extra-mural posts have been established. At others the subject is taught by scientists, philosophers or education department staffs. In Great Britain there is, as yet, no established honours school in the history and philosophy of science; but, in view of the value of the subject, it seems desirable that such a school be set up in the near future in one or more of the British universities.

The Australian universities are well ahead in the history and philosophy of science. Some of them are large in size, and they may have felt the need to introduce the subject in an attempt to solve problems relating to the integration of university studies. The Melbourne Department has a staff of six and two part-time tutors, and awards both honours and research degrees, as well as providing courses for other departments. Queensland and Sydney both have an established lectureship in the history of science. The Australian National University, Canberra, is contemplating making an appointment in this field.

Even among the smaller Canadian universities there seems to be an interest in the subject. In some of the university medical schools a course in the history of medicine is offered. At McGill University there is an established chair in the history of medicine, and Assumption University, Windsor, Ontario, has a professor of physics and the philosophy of science. There do not, however, seem to be any postgraduate or graduate degrees or diplomas available for interested students.

The overall picture in South Africa, at the moment, is that there are neither independent departments nor teaching posts. A fair amount of teaching is done by members of science departments, and there are a number of compulsory courses given at different universities. One University, Witwatersrand, apart from providing courses in the history and philosophy of science for B.Sc. students, has recommended the establishment of a teaching post.

Indian and Pakistani universities use the subject mainly for giving science students a historical background, and for broadening the interests of arts students. It is taught by lecturers in science and philosophy. Finally, there is a group of widely scattered universities and colleges—West Indies, Ibadan, Rhodesia and Nyasaland, and Hong-Kong—at which some instruction in the subject may be obtained. Out of these Hong-Kong seems to be the only one contemplating a separate course in the history of science.

[1] Mays, W., *Brit. J. Phil. Sci.* (November 1960).

THE CARNEGIE INSTITUTION OF WASHINGTON

ANNUAL REPORT

YEAR Book No. 59 of the Carnegie Institution of Washington* covers the year ended June 30, 1960, and includes the report of the president, Dr. Caryl Haskins (see *Nature*, March 4, p. 695), reports of Departments and Special Studies, as well as the administrative reports of the Executive Committee and the Auditors, and list of publications during the year. In his review of the year, Dr. Haskins again emphasizes the extent to which the growing-points of science arise at the line of contact between disciplines or depend on theoretical studies for guidance, so that fundamental and applied science really form a continuum. Growing-points have been particularly noticeable during the year in geophysics, the observation of outer space and of the stellar universe and in the study of the cellular structure of life and of its nature at the molecular level, and in all these fields the research workers of the Institution have contributed towards the understanding of our beautifully intricate and orderly world.

At the Mount Wilson and Palomar Observatories a cluster of galaxies, which are apparently receding from the Earth at about 138,000 km. per sec., was photographed with the 200-in. Hale telescope, and the cluster proved to be a strong source of radiation, particularly at the longer wave-lengths. It is estimated that the cluster is eight times as distant as the colliding galaxies in Cygnus *A*, and it is certainly several thousands of millions light-years distant from the Earth. Continued exploration of the interior of the Earth by the Geophysical Laboratory yielded results suggesting that the sharp transition in rock composition at some 400 km. below the Earth's surface may be at least partly due to a transition from one mineral form to another occurring naturally under the conditions of temperature and pressure at that depth. At high temperatures and pressures of 60,000–80,000 atmospheres, the iron–olivine fayalite has been metamorphosed into a dense spinel form, and since the olivines are very generally distributed within the Earth's crust and mantle, a transition from natural olivine to spinel may be a significant change associated with the structural discontinuity observed seismically at 400 km. Another feature of the year's work in this field was the synthesis of diamonds, while the opportunities implicit in the existence of the five independent nuclear age-dating methods continued to be explored, and new information was obtained which may help to resolve problems arising from the lack of agreement of ages obtained from zircon with those obtained from micas. A significant advance in the study of metastable phases and their relation to polymorphic transformations in sulphide minerals is noted, and an attempt to understand the structural mechanism of high-order transitions led to the conclusion that a mixed crystal is not a homogeneous edifice.

In the Department of Terrestrial Magnetism, a new 60-ft. radio telescope was installed which will be used to measure hydrogen clouds in our own Galaxy, and duplicates of the Department's extended antenna arrays for studying the radio emission of the Sun have been provided for collaborators in Chile, the Argentine and Uruguay, and, with assistance from the National Science Foundation, a duplicate of the Department's equatorially mounted parabolic antenna is to be built in South America, probably near Buenos Aires. Stress is laid on the Department's participation in the International Geophysical Year and afterwards in the International Geophysical Co-operation; but a major part of the scientific effort of this Department continues to be devoted to biophysics, particularly the study of protein synthesis by bacteria and other simple forms of life. During the year, ribonucleic acid was extracted from ribosomes in a manner that preserves hydrogen and magnesium bonds, and a unit equivalent to ribonucleic acid in a large ribosomal particle was obtained which could be broken down into units one-twentieth to one-fortieth of their size. These units were isolated from living cells and occur naturally both in the ribosomes and in the non-particulate fraction of the cells, and it also appears that the large ribosomal units are formed in the growing cell by assembly of these sub-units of ribonucleic acid and associated protein.

In the Department of Genetics, in work with the bacteriophage *T*2, which infects *Escherichia coli*, using chromatography on a column of basic protein, half molecules and quarter molecules of phage deoxyribonucleic acid have been identified, and the sedimentation coefficient and intrinsic viscosity of the quarter molecule point to a molecular weight of about 13 million. Evidence has also been obtained that formation of some proteins is at least linked with the development of phage deoxyribonucleic acid, and by comparing the effects of chloramphenicol when added at different times after the infection of bacteria with the *T*2 phage evidence was also obtained suggesting a correlation of the evolution of the resistance to radiation with the synthesis of deoxyribonucleic acid. Other work in the Department suggests that even mild chemical agents of cellular origin have mutagenic activity. The enzyme deoxyribonuclease proved mutagenic to *Drosophila melanogaster* under laboratory conditions, treatment of larvæ and imagines with deoxyribonuclease producing both recessive and dominant lethal effects and chromosomal re-arrangement in the flies.

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* Carnegie Institution of Washington. Year Book No. 59: July 1, 1959–June 30, 1960. Pp. lvii+515+25 plates. (Washington, D.C.: Carnegie Institution of Washington, 1960.) Cloth bound, 1.50 dollars; Paper bound, 1 dollar.

In the Department of Embryology, use of the techniques of rapid serial radiography and cinéradiography provided direct evidence that arterial blood enters the maternal placenta from the endometrial arteries under a pressure sufficiently high to drive it through the intervillous space and towards the chorionic membrane of the fœtus. Injection of radiopaque dyes into the femoral arteries of anæsthetized pregnant rhesus monkeys and observation of the course of the dyes by rapid serial X-ray photographs or by cinéradiography yielded films showing with great clarity the predicted characteristic spurts of blood as the dye enters the intervillous space. A major programme was completed in which chicken spleen was used to study the properties of immunologically competent tissues and the course of cells emanating from spleen grafts labelled with tritiated thymidine was traced within embryonic chick hosts. The cells of both donor and host appear to contribute to the spleen reaction, the host cells predominating numerically.

A new technique used during the year in the Department of Plant Biology facilitated the detection of the several forms of chlorophyll, and experiments with the algal genus *Phormidium* showed that the pigment phycoerythrin increased efficiency of light absorbed by chlorophyll *a* 695 and other forms of chlorophyll *a*. It also appears that a maximum rate of *Chlorella* photosynthesis is reached only when the two photochemical reactions initiated by the two parts of interdependent pigments are proceeding in the correct ratio. Studies of the amino-acids isolated from cultures of *Chlorella pyrenoidosa* and examination of their carbon isotope composition indicated that some of the amino-acids, such as leucine and valine, contain markedly less carbon-13 than is present in the inorganic environment, and while others, like serine, had ratios much closer to the inorganic carbon dioxide used as input, the carboxyl carbons of all chains contained a higher proportion of carbon-13 than the remainder of the carbon chains of the amino-acid. If it can be assumed that the isotope abundance found in the lipids or in the non-carboxylic portion of aspartic or glutamic acids in *Chlorella* is characteristic of the fixation of carbon dioxide in carbohydrates, a considerable fraction of the carbon fixed in *Chlorella* must be incorporated by pathways other than those traditionally described for photosynthesis.

Further progress is reported in the development of image tubes for telescopes, and while further technical refinement is still required to remove imperfections, thirty-fold reductions in exposure time have been obtained as compared with direct photography. Dr. Haskins's review directs special attention to a report by Dr. F. Chayes, of the Geophysical Laboratory, "Correlation in Closed Tables", included in that Department's report, as an example of uncommitted research in the Institution. The research was stimulated by certain problems in petrography, but has novel and potentially useful conclusions applicable to the statistical analysis of data obtained in many branches of experimental science.

TECHNICIANS IN ENGINEERING AND CHEMICAL INDUSTRIES IN BRITAIN

THE results of a sample survey of the employment of technicians in the engineering and chemical industries, carried out in 1960 by the Ministry of Labour, are analysed in the *Ministry of Labour Gazette* of December.

Replies received from 309 of the 400 firms approached, of which three-quarters employed more than 1,000 workers and the remainder 100–999, indicated that in most groups the percentage of qualified scientists and engineers to the total number of employees is a little higher than the corresponding percentages obtained from the survey of scientific and engineering man-power made at the beginning of 1959. Technicians formed 8·7 per cent of all employees of the firms included in the survey, the figure varying from 11·6 per cent in the electrical engineering group to 4·1 in the 'other metal goods' group. Except in the chemical and allied industries, few women technicians were employed, the proportion for all groups being 4·6 per cent, and for the chemical and allied industries 10·4 per cent; in the latter group, 34 per cent of the women were employed in research and development laboratories and 54 per cent on testing, inspection and analysis.

In the chemical and allied industries the proportion of technicians to qualified scientists or engineers was 1·6 : 1, compared with 4 : 1 in electrical engineering and electronics, and 7·6 : 1 in motor-vehicles and aircraft. The average ratio for all groups was 4·2 : 1, but the inquiry confirms that for engineering industries as a whole 5 or 6 technicians are required for each technologist. In all but the chemical group of industries the shortage of technicians in January 1960, measured by the percentage of vacancies that occurred at the time to the total number of technicians employed, was rather less serious than the shortage of qualified scientists and engineers a year earlier. The forecast demand for technicians in the two years following the survey was in most groups about twice the number of vacancies occurring. Over the whole field, the largest number (32·7 per cent) of technicians was employed in the design and drawing office, and 29·1 per cent of all technicians were design assistants or draughtsmen, testing, inspection and analysis accounting for 14·4 per cent.

Design and drawing office work accounted for 40·7 per cent of all vacancies for technicians (67·5 per cent in shipbuilding and marine engineering firms), but in the chemical industry 44·6 per cent of vacancies were for laboratory technicians for research and development. An analysis of requirements for the next two years presented a similar picture. In the chemical group of industries 63 per cent of technicians had some qualification, and this group employed 43 per cent of all technicians with the General Certificate of Education at Advanced Level, while in the shipbuilding and marine engineering industry 58 per cent of technicians had a qualification. About 10 per cent of all the firms referred to apprenticeships, technician, student or drawing office combined with courses at technical colleges for which release on one day a week was granted, but nearly 70 per cent replied that no courses of instruction were provided.

HEALTH OF BRITISH SCHOOL CHILDREN

"THE great majority of school children are not only robust and healthy, but are taller and heavier than their predecessors", states Sir John Charles, the chief medical officer of the Ministry of Education, in his report for 1958 and 1959*.

Sir John says under-nutrition has almost disappeared in Britain, and the differences between the heights and weights of children in good and poor districts are lessening. More fat than under-nourished children are being reported, but the number in each group is small.

Only about five per cent of school children contracted a notifiable infectious disease in the years under review. Tuberculosis continued to decline. Poliomyelitis reached its lowest level for thirteen years, and vaccination against it was undertaken vigorously everywhere. There were local outbreaks of diphtheria that involved 47 school children in 1958, of whom five died; seventy-seven were affected in 1959 with no deaths. Advocating the continued need for immunization the report directs attention to the fact that "as recently as twenty years ago 55,000 persons contracted diphtheria annually, of whom almost 3,000 died".

The main cause of death among children is through accident. More children aged 5–15 years die from accidents than from all congenital malformations and of circulatory, genito-urinary infections and respiratory diseases altogether. In 1958, 869 children of 5–15 years died from accidents, including 395 from accidents involving motor vehicles, 174 from drowning, and 58 from burns and scalds; 113 fatal accidents took place in the home.

The second chief cause of death of children of this age-group is cancer. There were 499 deaths in 1958, mainly from leukæmia. Other causes were respiratory diseases, 253; congenital malformations, 185; infectious diseases, 137; genito-urinary diseases, 76; and only 53 from diseases of the heart and blood vessels. More children died from appendicitis (44) than from rheumatic fever and rheumatic heart disease (30); these conditions were once widespread and deadly. The report stresses the need for unremitting research into the causes of the so-called congenital diseases and defects. "Medical research gives us reason to believe that some diseases that we consider hereditary or congenital in origin may well be found to be due to adverse influences in pregnancy or infancy." These diseases and defects cause the death each year of about 4,000 children under the age of fifteen years and leave thousands disabled. To this must be added those who are mentally retarded or who are blind or deaf from birth or early infancy. Although most children are healthy and without blemish, there are tens of thousands who are disabled to a greater or lesser degree.

The report welcomes the increasing number of education authorities which are considering, or who have already started, a modification of the fifty-year old system of periodical medical examination. In these areas all children will be examined after first starting and before leaving school; but, instead of a periodical examination of intermediate age-groups, doctors will visit the schools more frequently and give more attention to the new children who appear to need it. The hope is expressed that school doctors will be able to give more time to the study of particular diseases and disabilities as they affect children at school. Illustrations given are respiratory diseases—as many as 100,000 boys and girls have asthma some time during their childhood—and the nature and effects of skin diseases, from which tens of thousands of children are still reported to be suffering.

In 1958 more than two million children were examined at periodical school medical examinations. More than 809,000 were examined in schools and clinics at the special request of the parents, doctors, nurses, teachers, or others, and nearly 1,044,000 were re-examined for defects found at a previous examination. The comparable figures for 1959 were 2,138,000; 704,000 and 1,041,000.

About 15 out of every 100 children who were examined after first starting school were found with some defects which required treatment; in some areas, from one in five to almost one in two of these were not receiving it. Often the parents did not know of the defect or did not appreciate its significance. Prevalence of defects found at periodical inspections shows that 175,000 are treated annually for skin diseases; 200,000 orthopædic defects, mostly slight; 15,000 boys and girls are under treatment for epilepsy; 2,000 boys and girls of school age have diabetes. There are fewer children with heart disease. Of every thousand boys and girls examined, twelve had a heart defect in 1958 compared with eighteen in 1948. The recorded prevalence of lung disease in school children has become almost stationary since 1948. Of every thousand children examined 24 were found with lung disease in 1948 and 25 in 1958.

Although the number of verminous children is still "deplorably high"—219,000 in 1958 and 223,000 in 1959—it is less than half the figure of 464,000 in 1948.

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* The Health of the School Child: Report of the Chief Medical Officer of the Ministry of Education for the years 1958 and 1959. Pp. iii+231+8 plates. (London: H.M. Stationery Office, 1960.) 10s. 6d. net.

A small number of children attend school inadequately clad. There is, however, widespread comment about the unsuitable types of shoes worn by many children, especially older girls. A school doctor in Hertfordshire reported that younger children were well shod, but that "many girls of secondary school age would not be guided otherwise than by the dictates of fashion".

The report says that it is doubtful whether children will refrain from smoking so long as they see their parents and teachers enjoying the habit. A survey of about 3,500 boys and girls in two grammar schools and four modern secondary schools in a county borough near London shows that eight per cent of the children are 'regular' smokers—the smoking of five cigarettes a week being defined as regular for the purposes of the survey. One third of the children aged 14–16 who are 'regular' smokers smoke more than twenty cigarettes a week. Another survey carried out in Oxfordshire of about 8,300 children shows that nearly 1,600 are smokers and spend £4,350 a year on cigarettes. Nearly half these children say that they have heard that there is some connexion between cigarette smoking and lung cancer.

The report emphasizes that, generally, facilities are adequate for medical treatment, but are seriously inadequate for dental treatment; the national shortage of dentists is the main deficiency. The school dental service is able to treat only a little more than half the number of children who require treatment. A large number, many of whom attend non-maintained schools, are treated by the general dental service, but hundreds of thousands are neglected.

In 1959, for the first time, the number of part-time dentists did not balance the number of whole-time dentists who retired or who left the service.

Special surveys of the teeth of the 5–12 year old children in eight representative areas show that deterioration of the teeth was less in five-year olds, but greater in twelve-year old children between 1953 and 1958 than between 1948 and 1953. "It is significant that during the period 1948–1958 sugar consumption per head in the United Kingdom rose from 88·3 lbs. in 1948 to 103·1 lbs. in 1953 and 120·7 lbs. in 1958", states the report.

The report of the working party set up in 1949 "to direct and organize an investigation on the prophylactic effects of the local application of a solution of fluoride to school children's teeth", is published as an appendix to the report. The working party considers that the application of a fluoride solution to the teeth is not a worthwhile preventive measure in the conditions of the investigation, that is, when the treatment is given on a wide scale in the public dental service. This may well enhance the interest already being taken in alternative methods of caries prevention, such as fluoridation of drinking water and the dental health education of the public, and especially children, in such matters as proper diet and the cleansing of teeth.

A notable feature of the work of the School Health Service in the years under review is the increased attention given to children with emotional or behaviour problems, and also of those with speech defects, but there was a serious shortage of child guidance clinic staff and of speech therapists. Much attention was paid to handicapped children; 54 new special schools, with accommodation for 5,732 children, were opened in 1958 and 1959, bringing the total number of handicapped children in special schools to more than 60,000. Much interest, too, was taken in children with defective hearing, and an increasing number of local education authorities employed peripatetic teachers of the deaf to visit children in ordinary schools, or opened special classes in ordinary schools for partially hearing children. During this period a large number of the new Medresco transistor hearing aids were supplied to school children.

Sir John insists that a school health service cannot ignore the challenge of children from problem families, of those who have been before the courts, or of promiscuity among some older adolescents. These boys and girls formed a small minority of the school population, but they presented "a tough problem to the medical, education and social services—three services that must work together".

Appendixes to the report include, in addition to the usual statistical tables, a list of the research projects being undertaken in the school health service and an index to *The Health of the School Child* for the period 1935–57.

STRENGTH AND PROPERTIES OF TIMBER

RESEARCH into timbers is undertaken in England at the Forest Products Research Laboratory, Princes Risborough, and is under the auspices of the Department of Scientific and Industrial Research. *Forest Products Research Bulletin* No. 45, entitled "The Strength and Properties of Timber", by F. H. Armstrong*, supersedes two earlier ones on the same subject and contains fresh material. Information on the properties of no fewer than 172 kinds of timber is presented in the proportion of some 37 home-grown timbers to 136 imported timbers, figures which may appear to some to be rather disproportionate, but the Laboratory has done a useful service to Commonwealth countries while not actually ignoring home-grown material, which has been handled more intensively. The technique of timber-testing has made considerable advances in recent years, and one new development in England has been the adoption of the 'Monnin' system, which makes use of 2-cm. square instead of 2-in. square test-pieces, and is thus more suitable for small-diameter trees.

The gist of this *Bulletin* consists of three tabular statements occupying about 16 pages. The physical and mechanical properties of home-grown timbers are given in Table 1, those of imported timbers, based on 2-in. test-pieces, in Table 2, and those of imported timbers, based on 2-cm. test-pieces, in

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* Pp. vi+34+8 plates. (London: H.M. Stationery Office, 1960.) 3s. 6d. net.

Table 3. The contents of species in the last two tables do not conform. The data refer to the eight tests made, namely, six tests of physical properties, of (*a*) static bending, (*b*) impact bending, (*c*) compression parallel to grain, (*d*) hardness, (*e*) shear parallel to grain, and (*f*) cleavage, supplemented by physical tests of moisture content and specific gravity. These tests and the apparatus used are described in some detail with the help of photographs. Since the most important factors which influence the strength of a timber are its moisture content and specific gravity, these are dealt with in some detail. Regarding the latter, it appears that, in respect of the actual wood substance, it is for all practical purposes the same for all species, but that the amount of that substance varies with the species, so that there is, in fact, a wide variation of specific gravity, and strength, not only between different species but also within each species. The strength values given in the tables are primarily intended for a comparison of species. Here it must be pointed out that while the pieces tested may be representative of variation of the properties within individual trees or small groups of trees, they can scarcely be said to be fully, or even fairly, representative of any species for the whole range of conditions under which it is grown. The largest number of trees or planks tested refer to Weymouth pine (56), Corsican pine (38), beech (36) and Scots pine (35), whereas for some tropical species a single tree has provided all the test-pieces.

In this connexion the presentation of the data can be criticized on the grounds that the first letter of a name is not a good scientific basis for grouping, although it is admittedly useful in locating a species within a group. It does seem incongruous, for example, to find horse chestnut sandwiched in Table 3 between dahoma and North Bornean kapur. The grouping adopted here might have been taken a little farther; there would have been no disadvantage if the timbers of each country had been grouped together—in alphabetical order.

An interesting and unexpected feature of the *Bulletin* is an introduction to simple statistical methods useful in comparing results or testing relationships between variables, and justifiably applied here in handling the data from many samples from one tree or group of trees from one source. The recognition of the possible importance of such factors as capacity to resist fungous attack or the effects of atmospheric fluctuations seems to indicate a new outlook. The value of the graphs presented in Fig. 5, showing the relationship between specific gravity and maximum compressive strength, is much impaired by the absence of a key to link up the plotted numbers with the corresponding species.

This publication is a useful contribution to our knowledge of the properties of a wide range of timbers, many with very odd names, and it should be of value to importers and to timber-growers in Commonwealth countries who are endeavouring to find markets for so-called 'secondary' timber trees. To the layman it should convey some idea of the widely varying character of timber as a raw material.

DUCTILE BERYLLIUM

SOME fifty years of work on the production of ductile beryllium would appear to have come to full fruition by the announcement by the Chesterfield Tube Co., Ltd., a subsidiary of Tube Investments, Ltd., of its ability to supply this metal in a variety of sections, including finned tubes, with mechanical properties in the hot-extruded state, which include a tensile strength of 30–45 tons per sq. in. and an elongation in the longitudinal direction of 4–10 per cent*.

The problem of overcoming the brittleness of beryllium has been approached by controlling the purity of the metal, by reducing the grain-size and by control of the crystalline orientation. Although the two first avenues can exert beneficial effects, it is the last that, at present, offers the most promising line of attack. As a result of its hexagonal structure, beryllium shows marked anisotropy both of strength and ductility. At ordinary temperatures the main slip occurs on the basal plane and on the prism faces in the <1120> direction; the critical stress needed to initiate basal slip being about one-fifth of that on the prism face. Very little basal slip can, however, take place before fracture occurs, while quite extensive slip can occur on the prism face provided that the orientation is such that slip on the basal plane is inhibited. As the temperature is raised, the stress necessary to produce prismatic slip falls, and at 500° C. it is the same for both forms.

Mechanical operations such as rolling or extrusion result in a preferred orientation of the crystals to which hexagonal metals are particularly prone, whence the behaviour of extensively worked material approaches that of a single crystal. As a result, a tensile force applied normally to the basal plane results in a brittle fracture, but a force parallel to that plane leads to considerable ductility. In an extruded flat section, for example, the basal planes tend to lie parallel to the sides of the flat, the longitudinal ductility of the section being high, 5–15 per cent elongation, but transversely, the ductility may be negligible. The extrusion techniques which have now been developed control the orientation of the grains, so that in a tube the basal planes, instead of radiating from the tube axis, are randomly orientated over the cross-section. Bursting tests at 600° C. have shown 10 per cent expansion at failure for such randomly orientated tubes as compared with 2–4 per cent for tubes with the radial texture.

It would appear that, although beryllium with some degree of ductility has been known for some time, this is an important step forward which, moreover, may well have applications to other hexagonal metals, for example, magnesium.

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* Chesterfield Tube Co., Ltd. Fabrication of Beryllium Tubes and Sections: a New Structural Metal with Unique Properties. Pp. 16. (Chesterfield: Chesterfield Tube Co., Ltd., 1960.)

DEPOSITION OF FISSION PRODUCT NUCLIDES AFTER TWO SAHARA NUCLEAR TEST EXPLOSIONS

By Dr. V. HAVLOVIC

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THE daily concentration of the radioactive dust particles as short-lived radium and thorium daughter products and long-lived fission products from nuclear test explosions in ground-level air have been estimated by the filter sampling method over several years[1]. Continuous measurements of the radioactive fall-out and rain-out in weekly and monthly intervals were added to the previous methods later[2]. The influence of two Sahara nuclear test explosions (on February 13 and April 1, 1960) on the fission product nuclides in the ground-level air in Czechoslovakia was not characterized by a sudden rise of fission product activity, but by a slow increase during the second quarter of the year 1960. The beginning of this increase appeared at the end of February. This observation is in satisfactory agreement with the simultaneous measurements of the radioactivity in the fall-out and rain. The increase of the fission product nuclides in the ground-level air in the mean monthly values is evident: the mean fission product activity during the second quarter of the year 1960 was about 320 per cent higher than that during the fourth quarter of the year 1959 (= 100 per cent): the latter was the lowest during the whole year 1959 as a result of the stopping of nuclear test explosions on November 1, 1958.

During March 1–17, 1960, the fission product content of rain-water presented several high peaks as a result of the first French nuclear test explosion. After April 8, 1960, a new increase in the fission product nuclides in rain-water was observed as a consequence of the second Sahara nuclear test explosion. The radioactivity deposited on a 1-m.^{2} area was, for example, on March 1, 1960, 42 times greater than on February 27, 1960. The fission-product activity in the rain-water after the second nuclear weapon test showed no further peaks after the first one and its activity was much lower. The increase in the rain-water activity as a result of two Sahara nuclear tests lasted until June 1960. An interesting result is demonstrated by the mean monthly fission-product activities in the rain-water expressed as specific activities of 1 litre rain-water: if the mean activity in January 1960 is regarded as 100 per cent, February shows 98 per cent, March 795 per cent, April 244 per cent, May 170 per cent and June 77 per cent. The activities of the rain-water deposited monthly on a 1-m.^{2} area were remarkable too: if the mean activity in January 1960 is regarded as 100 per cent, February was 53 per cent, March 1,360 per cent, April 455 per cent, May 186 per cent and June 132 per cent.

Moreover, the deposition of radioactive dust which is removed from the atmosphere by wash-out in rain-water and by dry deposition directly on to surfaces was observed at weekly and monthly intervals by the pot-method. The weekly fall-out radioactivity results are presented in the histogram (Fig. 1). Two peaks may be seen there, the first one in the tenth week (February 29–March 6, 1960), the second one in the fifteenth week (April 4–10, 1960), as a result of the second nuclear test explosion.

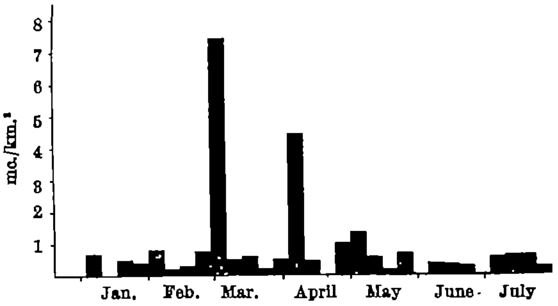

Fig. 1. Histogram of weekly deposited radioactive fall-out in the first half of the year 1960

Another method of autoradiographical research on the deposition of radioactive dust was tried in this laboratory independently of the macroscopical research methods described of observing fission-product nuclides in the ground-level air and in fall-out and in rain. This method rendered possible a microscopical observation of the deposited radioactive dust particles. For this purpose, glass plates (18 × 24 cm.) covered by a film of 'Vaseline' mixture were used. The sticky glass-plates were exposed for the deposition of the fall-out and rain in the free atmosphere for 14–21 days. Afterwards they were put for three days in a dust-free box for short-lived radium and thorium daughter products decay. Then the glass plates ready for autoradiography were covered with a thin plastic foil and pressed to a X-ray film (Agfa Laue film) for a longer period. The exposed X-ray film was developed in a hard-working developer.

The radioactive fall-out dust particles deposited, and fixed on the sticky surface, could be seen on the X-ray film after developing as dark centres of variable size and blackening. An interesting illustration of the quantity and quality of deposited radioactive fall-out dust particles on to the Earth's

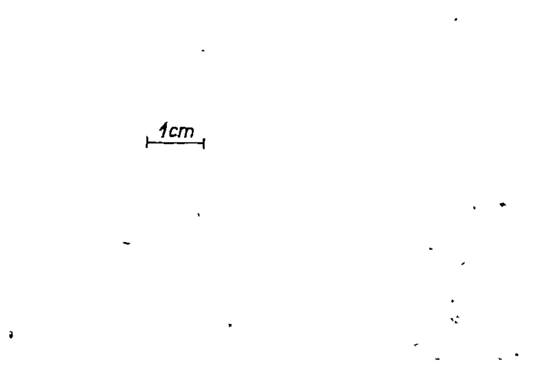

Fig. 2. Autoradiogram of radioactive dust particles deposited from the atmosphere from March 7 to March 20, 1960, on a sticky glass plate

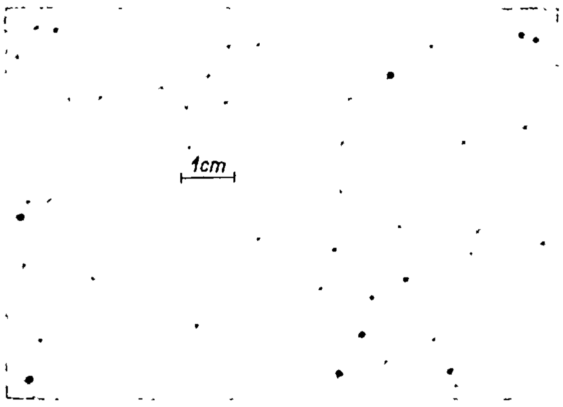

Fig. 3. Autoradiogram of radioactive dust particles deposited from the atmosphere from March 21 to April 10, 1960, on a sticky glass plate. It contains several 'hot' particles in consequence of the second Sahara nuclear test explosion

surface was given by the number and the blackening of centres.

Several glass plates exposed during the beginning of the year 1960, including that exposed in the ninth and tenth weeks (February 22–March 6, 1960), when a great peak in the weekly fall-out results was observed (Fig. 1), were not successful owing to a short autoradiographic exposure. The next glass plate exposed in the eleventh and twelfth weeks (March 7–20, 1960) gave an autoradiogram presented in Fig. 2. It contains only four radioactive centres of varying size and blackening. This result is in general agreement with the fall-out histogram in Fig. 1. The next glass plate exposed in the thirteenth to fifteenth weeks (March 21–April 10, 1960) is presented by its autoradiogram in Fig. 3. It gave a very clear result: it contains much deposited radioactive fall-out dust particles of different size and blackening. This autoradiogram demonstrates the presence and the deposition of a great mass of radioactive dust particles from the atmosphere, probably as a result of the second Sahara nuclear test explosion. This information is in good agreement with the second peak on the histogram in Fig. 1.

The further autoradiograms of glass plates, exposed in the free atmosphere in November 1960, contained several radioactive centres of small activities only. Thus the results described were verified, and an argument presented for a new purification of the atmosphere from the fission product nuclides in agreement with the results reached by other methods.

The deposited radioactive dust particles from the atmosphere create 'hot' particles with greater activity of the order 10^{-9} c. by agglomeration processes from smaller ones; several large centres in Fig. 3 may be due to 'hot' particles.

The concentration of fission product nuclides on dust particles has raised many new physical, biological and hygienic problems. As there is a possibility of inhalation of 'hot' particles or incorporation in some other way a radiobiological danger of irradiation arises, for example, on the endothelium of the bronchi.

[1] Havlovic, V., Patočka, St., and Matoušek, J., *Čsl hygiena*, 3, 391, 402 (1958), and work in the press.

[2] Havlovic, V., *Čsl. hygiena* (in the press).

A RADAR OBSERVATION OF A SEA-BREEZE FRONT

By Dr. E. EASTWOOD and G. C. RIDER

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IN the course of an investigation of the origin of radar 'angels', an unusual line source of radar echoes was recorded. Photographic time-compression techniques enabled the development and progress of the line echo to be investigated in detail. The photographic techniques and the parameters of the 23-cm. radar installation used have been previously described[1-3].

Fig. 1 shows the line echo as it appeared on the radar plan position indicator at 1700 hr. (6 p.m. B.S.T.) at about its maximum intensity, the isochrones on Fig. 2 indicating its development and movement. The line first became evident at about 1200 hr. along the coast near Felixstowe; it then moved inland, increasing in intensity and accelerating gradually until sunset, by which time it had reached nearly 50 miles inland and was moving at 6 knots. A similar line developed over Kent and Sussex, moving inland in a similar way. The whole phenomenon decayed rapidly over the sunset period 1930–2000 hr.

Scintillating discrete angels could be seen breaking away from the line during its strongest period; on the eastern side of the northern segment over Hertfordshire and Suffolk, and from both sides of the Sussex segments of the line.

In addition to the line echo the screen was well covered with discrete angels during this day. Most were of the short-lived, or rapidly scintillating kind, and these showed no very obvious pattern of movement. However, a well-marked stream of persistent angel echoes could be seen moving up the Thames estuary, and some of these could be tracked for considerable distances, occasionally even across the line echo.

The development of the line at the coast and the speed and form of its movement inland suggest a sea-breeze effect, and this appears to be confirmed by the meteorological evidence.

On June 20, 1960, an anticyclone covered the country, and there was almost no cloud and little wind. The data of Table 1, taken from the Daily Weather Report, indicate the changes over the afternoon at some relevant stations.

Table 1

Station	1200 hr. (G.M.T.)				1800 hr (G.M.T.)			
	Wind		Temp (°F.)		Wind		Temp.(°F)	
	Speed	Direction	Air	Dew pt.	Speed	Direction	Air	Dew pt.
Felixstowe	17 kt	50°	66	49	13 kt.	60°	62	51
Mildenhall	3 kt.	20°	60	52	5 kt.	40°	70	44
Gatwick	9 kt.	350°	71	51	6 kt.	90°	75	56
Thorney Island	4 kt	320°	73	45	2 kt.	200°	71	55

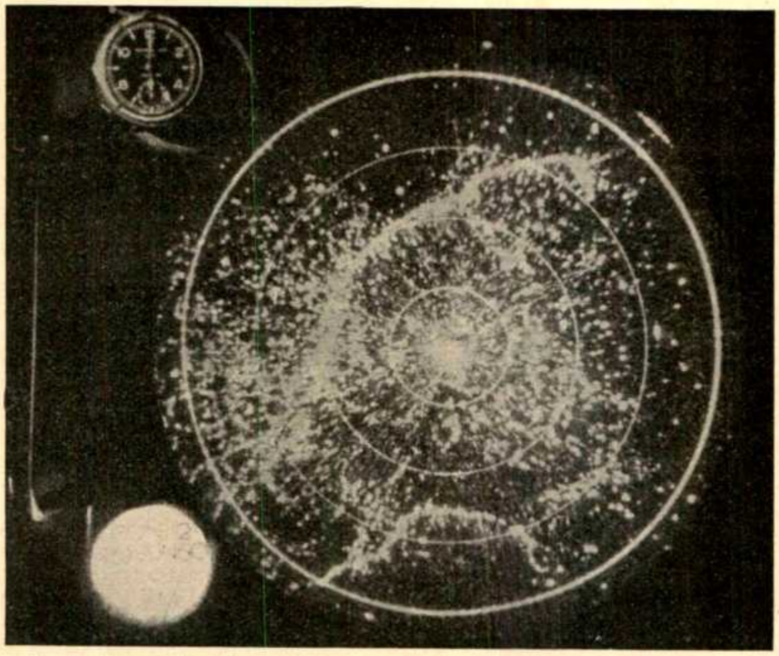

Fig. 1. Plan position indicator photograph, 1700 hr., June 20, 1960 (clock shows B.S.T.)

The line echo passed over Gatwick and would doubtless have been observed to pass over Thorney Island also if this station had been within range-setting of the radar display photographed. The changes in wind direction and in dew point at these two stations thus appear to offer good confirmation of the sea-breeze hypothesis.

It appears, then, that the radar was in fact seeing a remarkably well-developed sea-breeze front, for its maximum penetration inland was about 50 miles, while its greatest length, including both north and south segments, was little short of 120 miles.

It is of interest to consider the mechanism by which the radar energy has been reflected from the front. Cloud echo can be eliminated both by the plan position indicator appearance and by the 0/8 cloud reported by the relevant synoptic stations. The necessary echoing area must therefore be provided either by birds or by refractive index effects in the gaseous atmosphere. Formally, of course, both factors certainly contribute, and in this case it will be shown that each can make a significant contribution.

The discrete angel echoes moving away from the line echo have already been mentioned. They scintillate strongly, and move from the line at a velocity of about 30 knots, though the scintillation makes accurate measurement difficult. As discussed below, the frontal zone is a region of strong convection, so that soaring birds may well be present along the line echo, and may account for the angels breaking from it, since the velocities measured are of the correct order. A small flight of birds would provide sufficient echoing area to account for these angels, and if many such flights were air-borne, soaring in the frontal region, their resultant echo would present the continuous appearance seen, as in the case of the ring angels already shown to be due to starlings[2]. The sharp edge seen in the frontal echo is also evident in the rings.

We now consider the magnitude of the echoes to be expected from the refractive discontinuities.

Excepting the front under discussion, the synoptic situation was almost stationary throughout the day, and the radiosonde ascent from Hemsby at 1100 hr. gives adequately representative data. The atmosphere was very stable and abnormally dry to 1,400 m., and the refractive index profile shows a step discontinuity of 13 N units at this level ($N = (n - 1) \times 10^6$; n being the refractive index coefficient). The slope of the N profile is otherwise as for a standard atmosphere. The refractive index of the sea-breeze air may be estimated as follows. Air having the moisture content shown by Felixstowe at 1800 hr. (dew pt. 11° C.) and warmed to the temperature shown by Cardington (22° C.) is transported adiabatically to 1,400 m., at which height a difference of 30 N units is shown with the ambient air.

The frontal region is thus considered as a line of thermals rising to about 4,000 ft. In the absence of mixing the rising air differs from its surroundings by 30 N units at this level.

If this discontinuity can be considered sufficiently sharp, taking place, for example, in about 5 cm., then a plane reflexion coefficient:

$$\begin{aligned} \rho &= \tfrac{1}{2}\,\Delta n \\ &= 1{\cdot}5 \times 10^{-5} \text{ may be used (ref. 4, p. 7)} \end{aligned}$$

If the beam is filled with such a sheet, from 4,000 ft. to say 1,000 ft. at ground cut-off, the signal received may be calculated to be 1 db. below the threshold of detection.

The actual target configuration is not a plane sheet, however, but numerous discrete thermals, rising somewhat 'doughnut' shaped; for example, as described by Scorer[5]. Each thermal thus presents

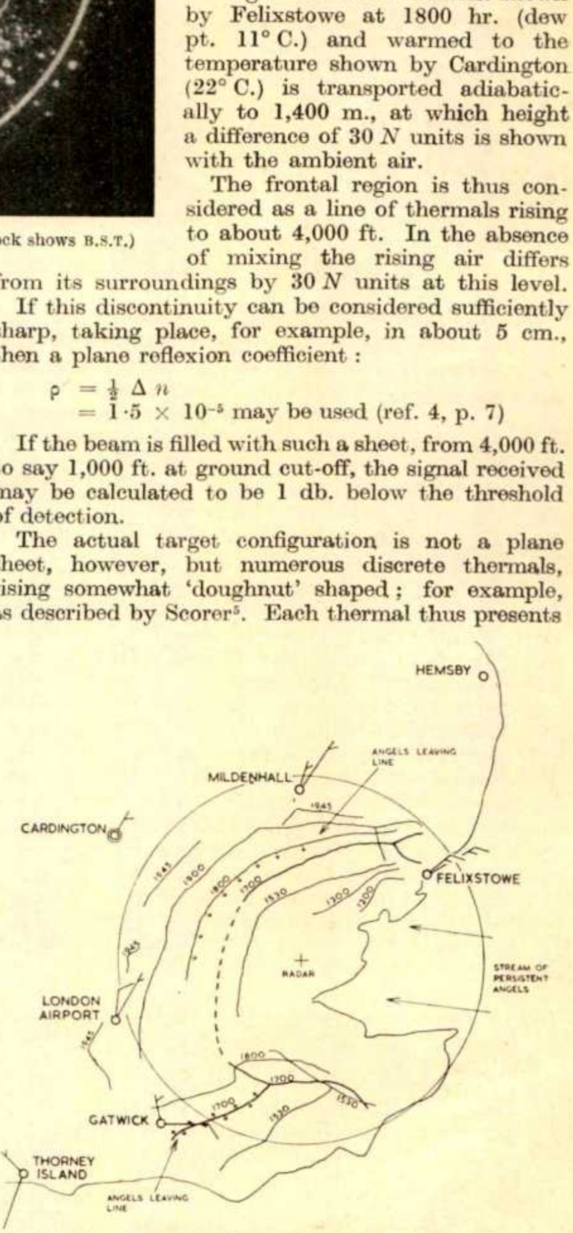

Fig. 2. Development of the sea-breeze front, June 20, 1960. Range ring 40 nautical miles. Surface winds: 1200 hr., short arrow; 1800 hr., long arrow

the discontinuity to the incident energy many times, and though the intensity of the reflecting boundary must decay due to mixing, there is also the possibility of focusing of energy from the rear surface of the thermal column. It appears quite possible that such thermals, probably filling the pulse volume in depth, may provide a returned signal greater in intensity than the hypothetical plane sheet used in calculation.

Rather similar line echoes have been reported by Atlas[6], and by Ligda and Bigler[7], each favouring a refractive explanation similar to that offered above. In the former case, using 1·25-cm. radar, the absence of birds was verified. However, Harper[8] has produced evidence in favour of soaring birds as the backscatterers responsible for some line echoes of somewhat similar appearance.

In conclusion, we comment on the length and penetration inland of the sea-breeze front observed, and secondarily note that in this case it seems probable that both birds and refractive discontinuities have contributed to mark its passage.

We thank our colleagues, especially in the Radar and Photographic Groups, who made the observations possible.

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DIFFUSION THROUGH POROUS MATERIALS

By J. R. BELL and Dr. P. GROSBERG

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It is often necessary to calculate the amount of a gas or vapour diffusing through a porous bed of material. The diffusion is less than that through the air because of the interposing solid portions through which, in general, there is virtually no diffusion. Usually an attempt is made to relate the ratio D/D_0 to the porosity ε, where D is the diffusion coefficient for diffusion through the porous structure, and D_0 is the corresponding coefficient through air. For example, in the later stages of the drying of thick porous structures the water surface retreats inwards and the falling rate of drying is maintained by the diffusion of water vapour from the surface of evaporation through a layer of the dry material. In calculating this diffusion, Nissan, Kaye and Bell[1] made the simple assumption that, with no diffusion through the solid, the area available for diffusion would be the average area of the air space, that is, εA, where A is the total area of cross-section. This means that D/D_0 is equal to ε. This relationship was adequate to obtain the diffusion through highly porous textile structures, but for less-porous textile materials the amount of diffusion occurring is much less than that obtained in this manner.

If one considers the case of diffusion through a highly porous bed made up with solid spheres and compares it with the analogous case of conduction, the picture is that of conduction through a continuum of good conductor with spheres of insulator interspersed in the good conductor. Maxwell[2] gave the following equation for these conditions:

$$k = k_s \frac{1 - \left(1 - a\dfrac{k_s}{k_p}\right) b}{1 + (a-1)\,b}$$

$$\text{where} \qquad a = \frac{3k_s}{2k_s + k_p}, \quad b = \frac{v_p}{v_s + v_p}$$

where k_s is the conductivity of the solid—the good electrical conductor; k_p is the conductivity of the pores—the spherical air spaces in the good conductor; v_p and v_s are the relative volumes of pore and solid.

For the case of diffusion the suffixes must be interchanged, that is, the interspersed spheres are the solid—thus $b = (1-\varepsilon)$. Assuming the diffusion through the solid is zero, this leads to $D/D_0 = 2\varepsilon/3 - \varepsilon$. In Fig. 1 experimental data due to Penman[3] for particulate systems are plotted, and it can be seen that curve B based on Maxwell's equation gives the general shape of the curve followed by the experimental points. Penman suggested that $\dfrac{D}{D_0} = \dfrac{2}{3}\varepsilon$, and it can be seen that, when ε is small, the above expression tends to this value. Maxwell's equation applies strictly only for high porosities, but it nevertheless shows good agreement with points from experiments using beds of glass spheres and beds of sand where the porosity is quite low. The line $D/D_0 = \varepsilon$ has been drawn on Fig. 1 and gives a first approximation to the results.

If one considers diffusion through the gap between two parallel solid cylinders, values of D/D_0 may be calculated for various spacings of the cylinders. A relaxation method has been used to compute such values. When the cylinders are interwoven by other cylinders perpendicular to them the porosity can be

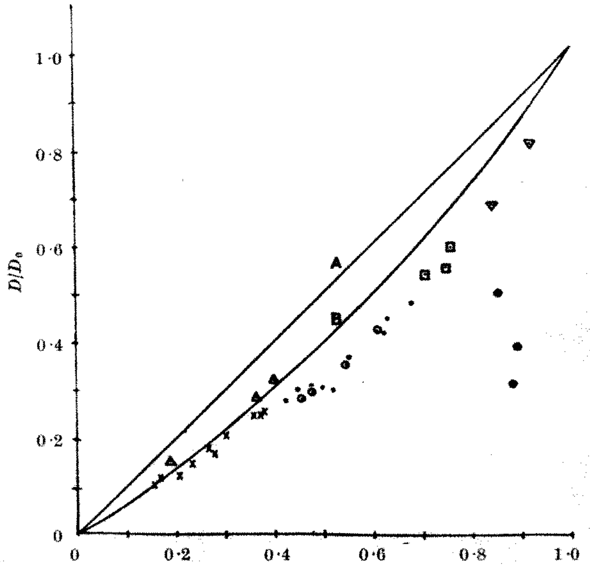

Fig. 1. Diffusion through particulate systems. Penman (ref. 3). △, glass spheres; ×, sand, sand mixture; ○, salt; ●, soil; ⊡, talc; ▽, kieselguhr; ⊗, mica
A, $D/D_0 = \varepsilon$; *B*, $D/D_0 = 2\varepsilon/3 - \varepsilon$

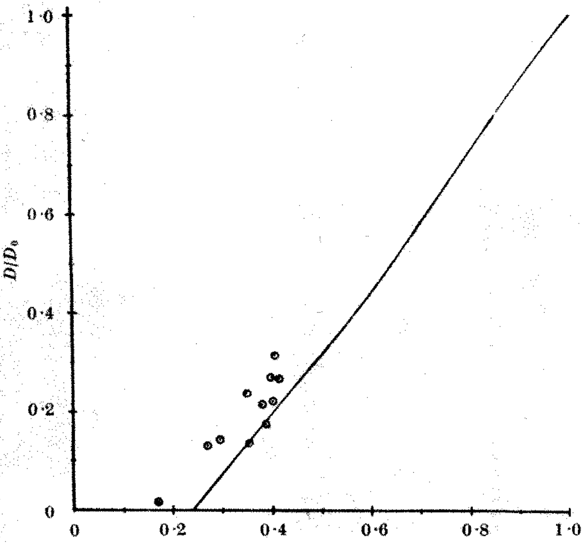

Fig. 2. Diffusion through leather. Robinson and Topp (ref. 5). ⊙, experimental; —, theory

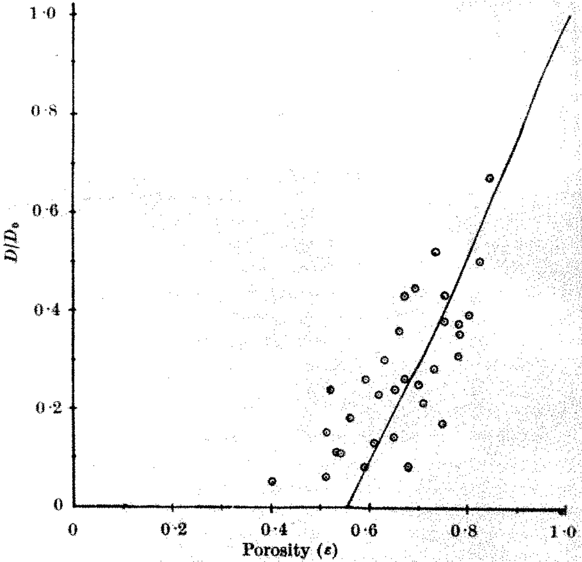

Fig. 3. Diffusion through textiles. Fourt and Harris (ref. 6). ⊙, experimental; —, theory

calculated as shown by Pierce[4], who considered such a geometrical configuration in connexion with textiles. In Fig. 2 the curve relating D/D_0 and the porosity obtained in this manner is given. Some experimental data due to Robinson and Topp[5] for the diffusion of water vapour through leather are compared with the curve, and it can be seen that there is reasonable agreement. The collagen fibres in leather are interwoven so that the model used is a suitable one. When the parallel cylinders touch, diffusion is no longer possible but there is still a value of porosity. Thus the curve has an intercept on the porosity axis and differs in this respect from the curve for spheres (Fig. 1)—when the spheres touch there is still a path around them for diffusion. In a woven structure the parallel cylinders cannot touch along their entire length and thus there will always be some diffusion. This is probably the reason why the experimental points for leather are to the left of the curve.

So far as textiles are concerned the model fits the case of the woven yarns. The fibres in the yarns are twisted together so that a direct path through a yarn via gaps between fibres is unlikely. If one assumes that there is no diffusion through the yarns and applies the case of the parallel cylinders to the yarns only, then the curve given in Fig. 3 results. The values have been obtained using the average porosity of yarns found by Pierce to apply to all cotton yarns in woven fabrics. Experimental data for textiles due to Fourt and Harris[6] is compared with the curve and it can be seen that there is again reasonable agreement.

It has thus been possible to obtain values for diffusion through porous materials from theoretical considerations even in the case of textile structures. These latter have often given anomalous results when suggested equations for permeability have been applied to them.

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GENETIC POLYMORPHISMS IN THE EGG ALBUMEN PROTEINS OF THE DOMESTIC FOWL

By I. E. LUSH
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RECENTLY, Sibley and Johnsgard[1], using paper electrophoresis, indicated that they had observed differences in the protein 'profiles' of egg albumen between hens of several breeds. With starch gel[2] it has been possible to establish the existence of consistent differences between the electrophoretic patterns of the albumen proteins of individual hens from sixteen small closed populations. It has further been found possible to classify these differences and to accumulate sufficient genetic results for a tentative explanation of their inheritance.

The thin albumen from eggs which had been kept up to four weeks at 4° C. was drained into muslin-covered beakers and then used for electrophoresis without any treatment, absorbed on to filter paper inserts. The storage had no effect on the electrophoretic analysis. The discontinuous buffer system of Poulik[3] was used, slightly modified (gel: 0·076 *M tris*, 0·005 *M* citric acid; *p*H 8·65. Electrode vessels: 0·3 *M* boric acid, 0·1 *M* sodium hydroxide *p*H 8·81). Gels were stained overnight with 0·005 per cent nigrosine or with 1 per cent naphthalene black 12*B* for 15–30 min., and photographed by transmitted light. Nigrosine reveals the pre-albumins better than does naphthalene black.

Eggs from the sixteen closed populations listed in Table 1 were investigated. All except the Bush Crossbred population have been subjected to some degree of selection or inbreeding, or both. The Bush Crossbred population is the F_2 generation of a 4-way cross involving commercial birds of four breeds; White Leghorn, Rhode Island Red, Indian Game and Light Sussex. The Jungle Fowl have been maintained as a small flock of 12–15 birds for about eight years at the Ministry of Agriculture Veterinary Laboratory, Lasswade. The Wicob White Leghorns are a flock of very heterogeneous origin kept at the School of Agriculture, Cambridge. The brown Leghorn, *Q* and Bush Crossbred are fed on Poultry Research Centre (P.R.C.) breeder mash[4], and the others on comparable standardized diets.

The Albumen Phenotypes

Considering the electrophoretogram as a whole, all but one of the 470 hens tested during a period of six months lay eggs that fall into one or other of nine distinct albumen phenotypes. 67 hens have been tested twice and 6 hens six times or more, and all except one hen described below have been consistent in their egg albumen phenotype. The albumen types differ in one or more of three separate regions of the electrophoretogram, designated I, II and III. Fig. 1 is a starch-gel electrophoretogram, which has been stained with naphthalene black, of albumen from five different eggs which between them show all the elements of variation that have been found. Sample 1 is from the Large Egg population, samples 2 and 3 from Red Plumage, sample 4 from Breeding, and sample 5 from Bush Crossbred. The wide range of intensities of the stained fractions makes photography unsatisfactory and a clarifying diagram has been added.

The two pre-albumins visible with nigrosine staining are marked in Fig. 1. One is barely separated from the main ovalbumin complex and the other lies along the brown buffer boundary referred to by Poulik[3].

Region I. Ovalbumin has been shown to consist of three components differing in phosphorous content[5]. The question of whether the variation to be described in region I of the gel involves ovalbumin itself or one or more closely adjacent proteins is left open by the use of the purposely non-committal symbol I. The same argument applies to the other regions in which the pattern varies, in both of which the particular protein involved is unknown. Region I presents two alternative variants. In I.*A*, fractions 6 and 7 are present and fraction 5 cannot be detected. In I.*b*, fractions 6 and 7 are absent, fraction 5 clearly present and the main ovalbumin complex of fractions 3 and 4 travels slightly faster than in I.*A*, although this last effect is sometimes obscured by distortion of the

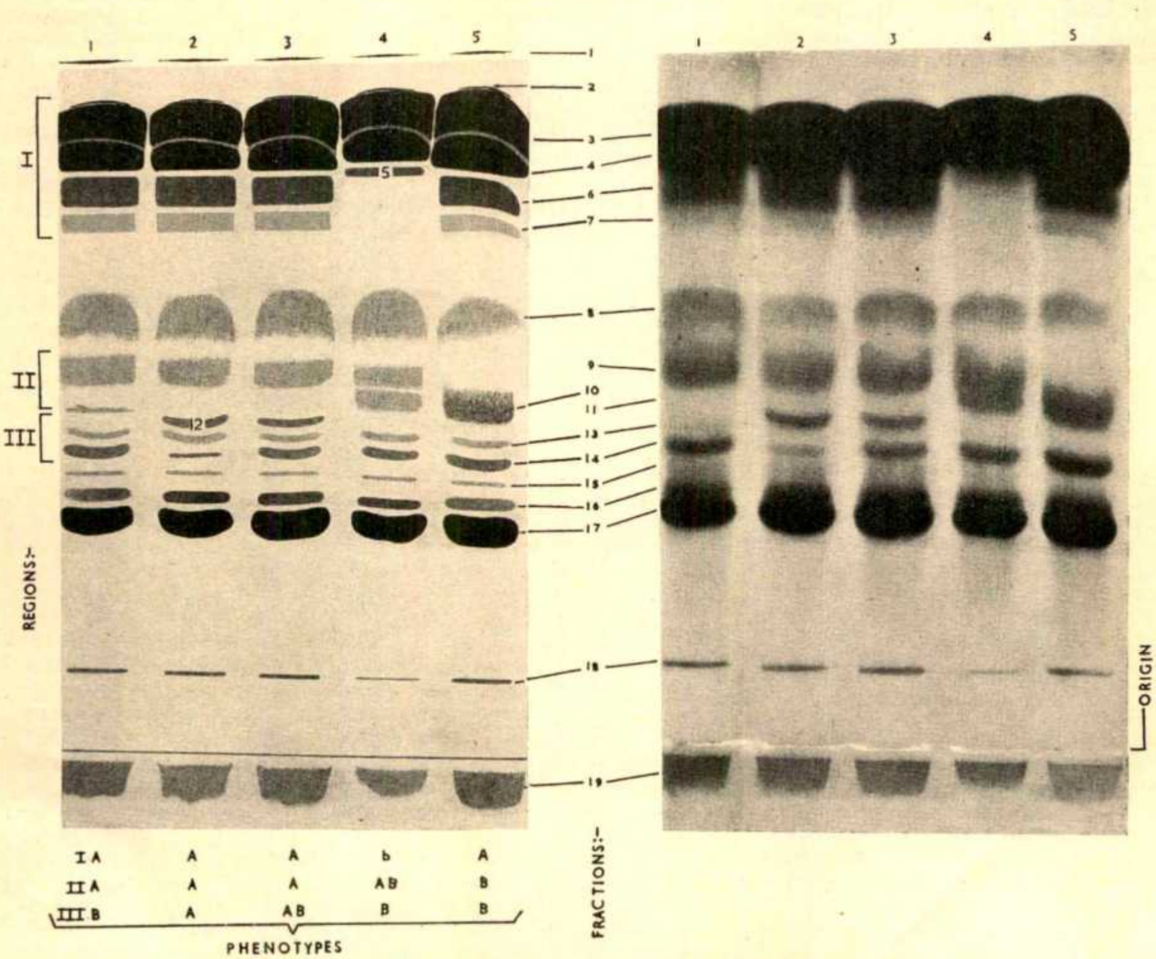

Fig. 1. Starch-gel electrophoretogram of representative egg albumen phenotypes

Table 1. DISTRIBUTION OF EGG ALBUMEN VARIANTS IN PRESENT GENERATION OF HENS FROM 16 CLOSED POPULATIONS

Population	Regions of electrophoretogram								Co-efficient of in-breeding (per cent) *F*
	I Variants		II Variants			III Variants			
	A	*b*	*A*	*AB*	*B*	*A*	*AB*	*B*	
Brown Leghorn P.R.C. selected lines									
1. Breeding *B.*	19	56	53	22				75	31*
2. Large egg *L.*	34		34					34	64*
3 Intensity *I.*	38	4	42					42	46*
4. White plumage *W.*	10	4	14					14	62*
5. Red plumage *R.*	38		38			23	15	(3)†	74*
6. Non-moult *N.*	27		27				1	26	57*
7. Small egg *S.*	7		7			6	1		79‡
8. Dwarfbody *D.*	13		13				7	6	58†
White Leghorn Reaseheath in-bred lines									
1. *RE*	25		25			25			98 63
2. *CH*	18		18					18	99 62
3. *IA*	20		20					20	99·57
4. *WA* (cuckoo Leghorn)	12		12					12	99 11
Cambridge Wicob	22		22				3	19	—
Rhode Island Red *Q* Inbred line	9		1	3	5		2	7	92 55
Bush crossbred	12		5	4	3		1	11	—
Jungle fowl	2		1	1				2	—

* Coefficient of inbreeding computed up to 1958.
† Surviving members of a previous generation.
‡ Coefficient of inbreeding computed up to 1953.

electrophoretogram. In Fig. 1, sample 4 is I.*b* and the rest are I.*A*.

Region II. Three variants have been found, involving fractions 9 and 10. In II.*A*, fraction 9 is present and fraction 10 is absent, in II.*AB* both fractions are present, and in II.*B*, fraction 9 is absent and fraction 10 present. In Fig. 1, sample 5 is II.*B*, sample 3 is II.*AB* and the rest are II.*A*. The diffuseness of fractions 9 and 10 makes it difficult to judge their intensity of staining by eye, but within a population that contains all three variants both fractions seem more strongly stained when they occur alone than when they occur together.

Region III. This also shows three variants. In III.*A*, fraction 12 is strongly present and fraction 14 is faint. In III.*AB* both are fairly strong, with 14 usually slightly stronger than 12. In III.*B*, fraction 12 is absent and 14 strongly present. In Fig. 1, sample 2 is III.*A*, sample 3 is III.*Ab* and the rest are III.*B*.

Several of the protein fractions have a very distinctive shape and character. Fraction 11, which tends to be obscured by 10 and 12, is rather variable in position but usually runs just ahead of fraction 12 and never behind it. The amount of some of the fractions, as judged by staining intensity, appears to be subject to quantitative genetic variation between populations, subsidiary to the phenotypes described above. For example, in Intensity and Bush Crossbred eggs, fractions 9 and 10 are typically stronger than in eggs from elsewhere, and in the Bush Crossbred eggs fraction 14 was also unusually strong (Fig. 1, sample 5). Within the Breeding line fraction 18 is weaker in some individuals than in others (Fig. 1, sample 4). Wilcox and Cole[4] found that the lysozyme concentration in egg albumen is amenable to selection both up and down, so quantitative differences are to be expected. The age of the hen and the position of the egg in the clutch seem to be without effect on the albumen phenotype. The first three eggs of one Large Egg × Breeding F_1 pullet gave a typical I.*A*, II.*A*, III.*B* pattern.

The distribution of the regional variants in the current generation of hens from sixteen closed populations is listed in Table 1. Of the nine phenotypes found so far, five can be read directly from Table 1 in those populations that vary only in one region, two others are recorded in Table 3 and the remaining two (I.*A*, II.*AB*, III.*AB* and I.*A*, II.*B*, III.*B*) were both present in the Bush Crossbred and Q populations.

Genetical Data

The genetical data suggest that the variation at the three regions is caused by segregation at three corresponding loci, two alleles segregating at each locus.

In region I the hypothesis of two alleles at a sex-linked locus was at first suggested to explain the two variants, but it had to be abandoned in the face of the pedigree from the Intensity line summarized in Table 2. If the locus is sex-linked, then in order to produce offspring both I.*A* and I.*b* the sire (being the homogametic sex) must be heterozygous, and the two variants should be present in approximately equal numbers. On this hypothesis the observed distribution would be very improbable ($\chi^2 = 9{\cdot}39$, $P < 0{\cdot}005$). Unfortunately, the dams of the two I.*b* offspring died too soon to be tested, but the argument is still valid. Several I.*A* and I.*b* dams have produced daughters of both types. Therefore locus I cannot be borne on a *W* chromosome.

Table 2. EGG ALBUMEN PHENOTYPES IN A HALF-FAMILY FROM THE BROWN LEGHORN INTENSITY LINE

Sire	Phenotypes of dams	Phenotypes of offspring	
		I.*A*	I.*b*
Cock. *G.* 3839	(dead)	3	
	I.*A*	5	
	I.*A*	4	
	(dead)	1	1
	(dead)	3	1

Since only two variants have been seen in region I, an autosomal locus with one allele dominant over the other must be postulated. It will be shown here that the allele determining the I.*A* variant is dominant over the allele determining the I.*b* variant, and it is proposed that the dominant allele be symbolized by I^A and the recessive allele by I^b. The heterozygote, I^A/I^b, hen would then lay eggs of type I.*A*.

F_1 hybrids between several of the Poultry Research Centre selected lines have been produced in the course of a programme of work on heterosis in this laboratory. Of these, 34 F_1 hens from the following crosses have been tested: Breeding × Large Egg, and its reciprocal; Breeding × Red Plumage, and its reciprocal. All 34 hens laid eggs with I.*A* albumen. If I^A is dominant over I^b, then I^b can only be present in the Red Plumage and Large Egg lines in heterozygotes, in the cocks, or in the untested hens (less than 5 in either line), and I^A must be the more frequent allele in these two populations. It would then be expected that most of, or even all, the hybrids with Breeding would be phenotypically I.*A*, which they are. If I^b is dominant over I^A, then the absence of I.*b* hens among the hybrids is inexplicable. 8 of the hybrids came from Breeding dams known to be I.*b*. If I^b is dominant, these dams must be heterozygotes and their progeny should approximate to a 1.I.*A* : 1.I.*b* ratio. The

Table 3. Distribution of Egg Albumen Phenotypes in the Brown Leghorn Breeding Population

Pooled data from the current generation and the surviving members of the three previous generations

Variants	Variants II.*A*	 II.*AB*	 II.*B*	Totals
I.*A*	26	5	0	31
I.*b*	48	27	2	77
Totals	74	32	2	108

observed absence of I.*b* hens among the 8 hybrids makes this hypothesis untenable ($\chi^2 = 6{\cdot}12$, $P < 0{\cdot}02$).

In region II, fractions 9 and 10 can be considered separately or together. If separately, it can be postulated that the presence and absence of fraction 9 are determined by two alleles at one locus, one allele being dominant over the other. Fraction 10 could be determined in the same way at a different locus. If together, it can be postulated that each of the two protein fractions is the product of a different one of two alternative alleles at only one locus, locus II; in other words, a one gene equals one fraction (= one protein) relationship. The available data are compatible with any of these hypotheses, but the last seems most acceptable for the following reasons. No egg albumen which lacks both fractions 9 and 10 has been found. Fractions 9 and 10 resemble each other in shape and diffuseness, and differ in these respects from most of the others. Finally, as was mentioned before, when both fractions are present there seems to be less of them than when they are present separately, so long as the comparison is made within a population. If the single-locus hypothesis is confirmed, the alleles determining the presence of fractions 9 and 10 may be symbolized by II^A and II^B respectively.

Table 3 shows the distribution of phenotypes in the Breeding line, both the present generation and the surviving members of the three previous generations. There is a slight but not significant association between I.*A* and II^A ($\chi^2 = 5{\cdot}01$, $P > 0{\cdot}05$). In both the present and the pooled Breeding line data the distribution of the region II variants does not differ significantly from a Hardy-Weinberg distribution.

In region III the genetical data and the electrophoretic variation resemble region II and the same arguments apply, with one complication. Let us postulate a locus III at which two alleles, III^A and III^B, determine the presence of proteins 12 and 14 respectively. In the homozygote III^B/III^B, only protein 14 is synthesized, in the heterozygote III^A/III^B both proteins are present, and in the other homozygote III^A/III^A only protein 12 should be present, yet in fact in variant III.*A* protein 14 seems to persist (see Fig. 1). However, there is no reason for supposing that the electrophoretic separation is perfect, and the anomaly would be explained if fraction 14 were shown to be a mixture of two genetically unrelated but electrophoretically superimposed proteins, the major one subject to the genetic variation, and the minor one constantly present in every egg throughout the population. This would also explain why in the heterozygote III^A/III^B (giving variant III.*AB*), when both proteins might be expected to be present in equal strength, fraction 14 nearly always appears stronger than fraction 12.

If, and when, chemical identities are given to the proteins involved in the variations described, loci I, II and III can be replaced by more informative symbols. Fractions 3 and 4 are almost certainly ovalbumin[7]. Unpublished work, in collaboration with Dr. W. N. M. Ramsay, of the Department of Biochemistry, University of Edinburgh, using iron-59, has shown that small amounts of added iron are bound almost exclusively by fractions 17, 16 and possibly 15, which can therefore be included in the term conalbumin. Fraction 19 is lysozyme.

It has been mentioned that one hen was not consistent in its egg albumen phenotype. This bird, *F* 3230 from the Poultry Research Centre Intensity line, hatched in June 1957, was used between April 25 and June 14, 1960, in a control experiment to test the effect of egg storage on albumen phenotype. All 12 of the eggs used were I.*B*, II.*A*, III.*B*, and it was noticed that the lysozyme fraction was unusually variable in amount. Between August 30 and October 6 the hen laid 8 eggs, and in all but the second of these fraction 12 was present but usually less strongly than in a typical III.*AB* variant. In the last two eggs, lysozyme had almost vanished and ovalbumin was becoming progressively weaker.

Nothing resembling this has been seen in any of the other several hundred eggs examined in this work, all of which have been readily classifiable without the slightest hesitation into one or other of the phenotypes described. The anomalous changes may be caused by a pathological condition in the oviduct.

This work was done during the tenure of a British Egg Marketing Board studentship. I am grateful to Dr. A. W. Greenwood for providing facilities and to Drs. J. H. Sang, D. J. Bell and J. S. S. Blyth for helpful discussions and advice. The Jungle Fowl eggs were kindly given by Dr. J. E. Wilson.

(*Note added in proof.* The above results were obtained with freshly made starch gels. With gels which have been stored overnight at room temperature, it has been found possible to discriminate between the albumens of hens of genotypes I^A/I^A and I^A/I^b. These findings will be described in detail elsewhere.)

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REPRODUCTION IN THE GREAT GREY KANGAROO

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TWO female great grey kangaroos (*Macropus major* Shaw) collected at Padthaway, south-east South Australia, in late October 1959, had a new corpus luteum in one or other ovary. At least one of these had recently mated, as spermatozoa were seen in glands of the sectioned uterus. In the same area females were pregnant, or had early pouch young, in late December 1959. At Blanche Town, South

Australia, females were pregnant in early November 1958. On Kangaroo Island, South Australia, the insular form of the great grey kangaroo (*M. major fuliginosus* Gould) was observed mating in late December 1956. At Padthaway, in October and December 1959, breeding females were suckling advanced young which had left the pouch, and in March and April 1960, females with hairless pouch young were suckling an older young out of the pouch. A single female, collected on Kangaroo Island in February 1958, had a hairless pouch young and a large young at heel which was being suckled. Three females in captivity at National Park near Adelaide, South Australia, were examined. In early November 1960, two were suckling young at heel and one of these had an early pouch young. The other gave birth to a young later the same month. The third National Park female had an early pouch young and was suckling a young at heel in mid December. In April 1958, at Blanche Town, and in August 1960, at Padthaway, females had large pouch young, but were not suckling young at heel.

The gestation period of the great grey kangaroo is 38–39 days[1]. The ages of pouch young, collected with their mothers in the wild, were unknown, but approximate ages were assigned by comparing them with young of known ages of the red kangaroo (*Macropus rufus* Desm.). New-born young of the two species are approximately the same weight (about 0·9 gm.) and the adults are approximately equal in size. It was assumed that growth-rates are comparable in the two species. All 11 Padthaway young examined were conceived between early October and early January and the three National Park young in early September and early and late October. The two Blanche Town young and the single Kangaroo Island young were conceived in November.

The observations on post-œstrous and pregnant females and on pouch-young indicate a breeding season from early September to early January. The great grey kangaroo breeds in December in northern New South Wales[2]. The association between mother and offspring is particularly long: suckling, in and out of the pouch, lasts at least 14 months in the Padthaway area.

Corpora lutea of pregnancy were reduced in size in March, two months after the end of the breeding season, and absent in August. However, three females taken in the breeding season had two corpora lutea of different ages. This indicates that the great grey kangaroo is polyœstrous, but I have so far been unable to determine the length of the œstrous cycle by taking daily vaginal smears of captive females.

Sharman[3] stated that lactation-controlled delayed implantation was a feature of reproduction in two species of macropod marsupials and probably occurred in at least three others. It is now known to occur also in the macropods *Protemnodon irma*[4], *Thylogale thetis*[5], *Macropus robustus*[6], *Lagostrophus fasciatus* and *Bettongia lesueur*[7]. In those species having delayed implantation that have been thoroughly investigated the gestation period is shorter than, but nearly equal to, the length of one œstrous cycle. During the period at which a quiescent, unimplanted blastocyst occurs in the uterus there is a small corpus luteum, formed at a post-partum ovulation and called the corpus luteum of lactation, in one ovary. Growth of the corpus luteum of lactation and development of the blastocyst occurs following premature loss, or removal of, the young from the pouch[3]. Female great grey kangaroos carrying young in the pouch did not have corpora lutea of lactation, and a female from which the young was experimentally removed, and which was known to have been with males at parturition, did not produce another young.

Females taken early in the breeding season at Padthaway and Blanche Town had new, developing corpora lutea in the ovaries and the uteri were in the proliferative phase. One of these, which had evidently recently mated, had the anterior vaginal canals and median vaginal culs-de-sac enlarged and filled with a creamy substance containing abundant sperm. In the others the occurrence of earlier mating could only be verified from the presence of sperms in the uterine glands. Similar conditions occur after œstrus and mating in other species of macropod marsupials[3]. Two females in late pregnancy, with embryos 19·3 and 22 0 mm. greatest length, had enormously distended anterior vaginal canals filled with creamy substance containing abundant sperm. The canals were less distended in a female with a new-born young (greatest length 21·5 mm.). No sperms were found in the vaginal canals of this female, but they occurred in the uterine glands. Copulation had thus recently occurred in these three females, but none had Graafian follicles containing mature eggs and none had recently ovulated. Mitotic activity was seen in the uterus of the female with the 22·0 mm. embryo but was not seen in the uteri of the two other females. The seminal fluid was evidently not left in the reproductive tracts from earlier inseminations because it was absent from females with earlier embryos measuring 9·5 and 19·0 mm. A little seminal fluid but no sperm was found in an animal with a 5·5 mm. embryo. No seminal fluid was found in the tract of one female with an embryo measuring 23·5 mm. greatest length.

It was concluded from these observations that delayed implantation is not a normal feature of reproduction in the great grey kangaroo as it is in related macropod marsupials. The œstrus which occurs near the end of pregnancy may be the result of possible high levels of extra-ovarian hormones secreted during pregnancy. As it is not accompanied by ovulation, it cannot be followed by fertilization as is the post-partum œstrus in related species. The possibility that this sterile copulation is the equivalent of post partum copulation in other species and that in the great grey kangaroo the gestation period has been extended beyond the limits of the œstrous cycle will be investigated next breeding season.

This investigation is based on a total of twenty-six adult females. Funds for the project came from Commonwealth of Australia Research Grants to Universities and from a generous grant from the Nuffield Foundation. The work was supervised by Mr. G. B. Sharman, who provided some of the specimens, with information on their history.

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ANALYSIS OF THE CROSS-REACTION BETWEEN TWO STRAINS OF TOBACCO MOSAIC VIRUS

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DIFFERENT virus strains are considered to be related antigenically if an antiserum prepared against each strain reacts in some way with the others. This combination, which is known as a cross-reaction, serves as the foundation for all strain relationship studies.

Although there is usually little or no controversy with the serological findings themselves, the symbolic representation of the results often do lead to dispute. This is understandable when one considers that cross-reactions may imply either of the following interpretations: (1) Each virus strain may be a mosaic possessing a number of distinct antigenic components. One or more of these components may be shared with other strains. Thus, a fraction of the antigenic surface may be identical on all strains displaying cross-reactivity, while the remaining antigenic components may be unique, and specific for particular strains. (2) Each virus strain may possess but a single kind of determinant which, however, is structurally similar to that found on related virus particles. In this case, strains are related if the spatial configurations of their surface antigens are similar to each other. They need not be identical to those found on related strains. These two cases are known respectively as the mosaic and single determinant case. They may, of course, be combined, that is, some sites may be identical on two strains while others may only be related. For our initial consideration here, however, the two cases will be treated as alternative extremes.

Frequently, in cross-reaction studies, it is not clear which of these alternatives the author has in mind, even though it is common for one or the other to be chosen almost arbitrarily. This is a problem which has been faced time and time again, and has been discussed at length by many investigators. For this reason, no attempt will be made in this article to review the subject of cross-reaction. The reader is referred instead to such general sources of information as Landsteiner[1], Kabat and Mayer[2], Race and Sanger[3], Burnet and Stanley[4], Matthews[5] and to an excellent summary of the problem from the immuno-geneticist's point of view by Owen[6]. The purpose of this article is to present a method of analysis which promises some success in distinguishing between the alternatives.

To a large extent, the difficulties encountered can be attributed to the reagents used for measurements, that is, the antisera. Both the mosaic and single determinant scheme require that the antisera which are produced, following immunization with single virus strains, are heterogeneous. The nature of this heterogeneity, however, is different for each case, so that two classes of antibody heterogeneity need to be considered.

In the first class, applicable to the mosaic case, discrete populations of antibodies are elicited, each of which combines specifically with the particular inciting antigen, and not with any of the others. These antisera are mixtures containing, at most, as many different kinds of antibodies as there are unique surface antigens on the virus used in the inoculum. Cross-reaction occurs when one of these discrete populations of antibodies combines with the surface of a related strain possessing an antigen identical to that of the immunizing strain.

In the second class, a single population of antibodies is present in each antiserum. These populations, although stimulated in response to single antigenic determinants, contain antibody molecules with broadened combining powers, capable of reacting with antigens that are structurally similar to the homologous one. The two antigenic cases and their accompanying antibody classes are summarized in Table 1.

Table 1. Possible Relationships between Two Virus Strains showing Cross-Reaction

Case	Strain	Antigenic surface	Antibodies
1. Mosaic	1	*A* ▨*B*	*a*, *b*
	2	*A* *C*	*a*, *c*
2. Single determinant	1	*A*	*a*
	2	A^1	a^1

This is the simplest example in which only two strains are analysed. Theoretically, for each new virus strain studied an additional antigen and corresponding antibody could be added under the mosaic concept whereas another antigen, again similar to A or A^1 would be assumed in the single determinant case.

For simplicity, the mosaic and the single determinant cases are described separately, although they need not be mutually exclusive. The two may well be represented in any one virus as already mentioned. In addition, the number of different antigenic determinants shown is the absolute minimum. Many more may be required to characterize a particular virus, depending on the complexity of the data and the number of different virus strains examined.

Although for practical purposes of virus identification and classification, it may be of little consequence to discriminate between the mosaic and single determinant scheme, the differences are nevertheless important. Each scheme leads to a distinct view both of the physico-chemical structure of the virus surface and of the amount of genic information required for the transmission of these characteristics. Thus, on theoretical grounds, the differences are significant enough to warrant all efforts to distinguish between the two.

Because of the long and successful history of the mosaic concept as applied to cellular and bacterial investigations, it is not surprising that this scheme

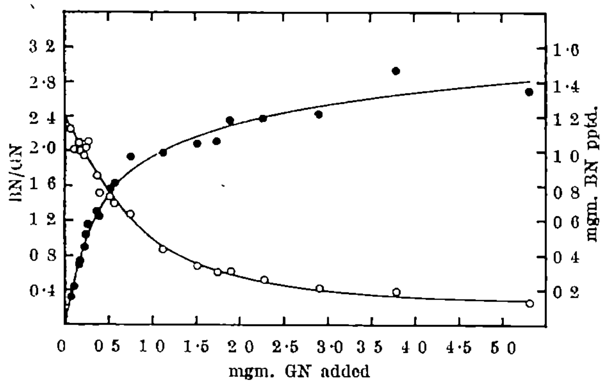

Fig. 1. Quantitative precipitin reaction. *U*2 vs. anti-*U*2 antiserum pool 17–18. Data are for antibody N precipitated in antibody excess to equivalence, and corrected to 1·0 ml. undiluted antiserum. Closed circles—antibody N (BN) precipitated. Open circles—ratio of antibody N to antigen N (BN/GN)

has played the dominant part in virus classification On the other hand, scheme 2, that of the single determinant, deserves more serious consideration, particularly because it has been demonstrated that a variety of immunological systems conform to the single determinant hypothesis. This by no means suggests that the mosaic hypothesis as applied to virus classification is inapplicable. It does, however, suggest that one observes some restraint in assigning a series of code letters to a virus strain in order to indicate its antigenic similarities or differences with related strains without the requisite evidence.

In those cases where the reactive sites are chemically defined the required evidence can be obtained, and proper relationships established, from a qualitative analysis of cross-absorption data. Many classical examples of such analyses are given by Landsteiner[1]. Unfortunately, the chemical nature of the antigenic determinants on the surface of virus particles are as yet undefined, and qualitative cross-absorption studies on a system with chemically unknown antigenic determinants are incapable of distinguishing between the two cases. Nevertheless, a quantitative analysis of the cross-reaction curves may resolve the issue. As a case in point, the quantitative data obtained from the cross-reaction between two related strains of tobacco mosaic virus, *U*1 and *U*2, will be described.

The homologous precipitin reaction for *U*2 and rabbit anti-*U*2 is shown in Fig. 1. The reaction is characterized by a steep rise in the amount of antibody nitrogen precipitated where the concentration of antigen nitrogen added is small. This region is followed by a more gradual and prolonged increment in antibody precipitated until the equivalence zone is reached. The ratio of antibody nitrogen (BN) to antigen nitrogen (GN) combined consequently falls precipitously at first, and then less so as more antigen nitrogen is added.

The more common equations for describing the quantitative precipitin reaction are inadequate to account for these data. Although other equations can be fitted to the data, they all suffer from the same shortcoming. They assume that only a single kind of antibody is combining with one kind of antigenic configuration. In the present case, we are concerned as well with the possibility of diverse antibody molecules reacting with a number of different antigenic sites on the same virus particle. For this reason, until proved otherwise, the precipitin curve shown in Fig. 1 is assumed to be a composite of at least two reactions, the sum of which adds up to the total shown.

Before proceeding with the analysis of the precipitin curve, it is well to point out that this tobacco mosaic virus/anti-tobacco mosaic virus system appears immunologically homogeneous according to Kendall's criterion[7]. A clear equivalence zone is observed where neither antibody nor antigen (or only minute traces of each) is detected in the supernatant liquid after the precipitates have been removed by centrifugation. This implies, by definition, that all *U*2 particles are identical with respect to antigenic characters and that there are present no detectable amounts of contaminant virus or other antigenic units and corresponding antibodies different from the bulk virus and antibody population studied in the reaction. This point cannot be over-emphasized. In those cases, for example, where it is demonstrated that different antigenic determinants are carried on distinct and separable molecules, the following analysis does not apply.

The Mosaic Case

Considering the mosaic hypothesis first, a resolution of the precipitin curve into two component parts can be accomplished if the following conditions be assumed :

(1) There are two discrete kinds of antigenic sites on the virus called *A* and *B*. Each type makes up some specified fraction of the total number of combining sites.

(2) Each type of antigenic site in turn stimulates the production of an antibody population which is specific ; so that anti-*A* (*a*) reacts only with *A* and anti-*B* (*b*) only with *B*.

(3) No interference is encountered during precipitation by either antibody type. The reactions of anti-*A* with *A* and anti-*B* with *B* occur independently of each other and progress to a final state which can be predicted from the concentrations of each antibody population and the two kinds of antigenic sites described in (1).

(4) In the presence of an excess of both antibodies, the maximum number of respective virus valences will be satisfied, and within this range of antibody excess the amount of antibody precipitated will conform to the quadratic equation proposed by Heidelberger and Kendall.

The observed shape of the precipitin curve imposes certain restrictions on the calculations of the fraction of antigenic sites assigned to *A* and *B* and on the concentration of the respective antibodies. With this limitation in mind the precipitin curve shown in Fig. 1 was resolved to a reasonable degree, by trial and error, into the components appearing in Figs. 2 and 3. This resolution is unique, for no other combination of two precipitin curves will satisfy the data better. From the maximum antibody-antigen ratios obtained in Fig. 3, the fractions of the total antigenic sites which are *A* and *B* are estimated. Approximately 0·83 (2 ÷ 2·4) are *A* and 0·17 (0·4 ÷ 2·4) are *B*.

In Fig. 2, it is seen that the amount of anti-*A* in combination with *A* rises steeply with small additions of virus, since most of the viral surface is *A*. With the addition of 0·55 mgm. of virus nitrogen all the anti-*A* is bound. From this point on, a constant amount of anti-*A* is found in the precipitate in addition to the increasing amounts of anti-*B*. The equation for the first part of the *A*/anti-*A* precipitin

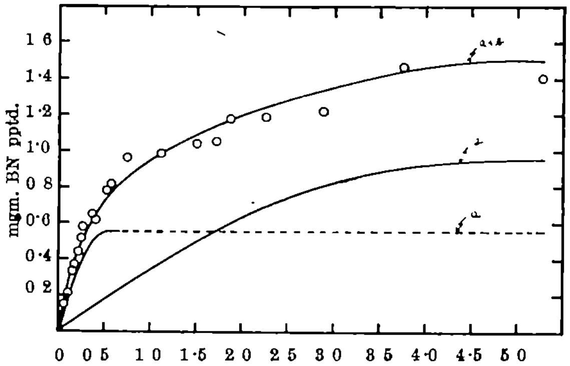

Fig. 2. Quantitative precipitin reaction. *U*2 vs. a hypothetical antiserum containing a mixture of antibodies *a* and *b*. The curves relate the amount of antibody N contributed individually by *a* and *b* to the precipitate and also the sum of the two. The open circles are the experimental points for antibody N precipitated taken from Fig. 1

curve is $\mathrm{BN}_A = 2{\cdot}0\ \mathrm{GN} - 1{\cdot}82\ \overline{\mathrm{GN}}^2$, where BN = antibody nitrogen precipitated and GN = antigen nitrogen precipitated.

The combination of *B* with anti-*B* progresses over a more extensive range since relatively little of the virus surface is *B*. Therefore, much larger amounts of virus nitrogen are required to bring the *B*/anti-*B* system to equivalence. At the point of equivalence for the *B* antigen the entire complex system reaches equivalence. Beyond this, further additions of virus will lead to an antigen excess zone. The equation describing the *B* system is $\mathrm{BN}_B = 0{\cdot}4\ \mathrm{GN} - 0{\cdot}042\ \overline{\mathrm{GN}}^2$, where the symbols have the same meaning as before.

The gross precipitation observed is the sum of the two quadratic equations to the point of equivalence for the *A* system, or $\mathrm{BN} = 2{\cdot}4 - 1{\cdot}86\ \overline{\mathrm{GN}}^2$. Beyond this point $\mathrm{BN}_{\mathrm{total}} = \mathrm{C}_A + 0{\cdot}4\ \mathrm{GN} - 0{\cdot}042\ \overline{\mathrm{GN}}^2$, where C_A is a constant equal to the maximum anti-*A* content of the antiserum. In this case $\mathrm{C}_A = 0{\cdot}55$ mgm.

Granted such a complex precipitating system, certain predictions can be made concerning any cross-reaction observed with this antiserum and related virus strains. The following cases are then logical consequences of the mosaic hypothesis :

Case 1. Both *A* and *B* are present on the test strain. In this case cross-absorption would remove all anti-*A* and anti-*B*. The test strain would be judged on this basis to be identical to the homologous. The test strain may not be identical in quantitative tests, if it were shown that either the ratio of *A* to *B* were different or the combined total of *A* and *B* was more or less than on the homologous strain, or that an antigen *C* was present in addition to *A* and *B*.

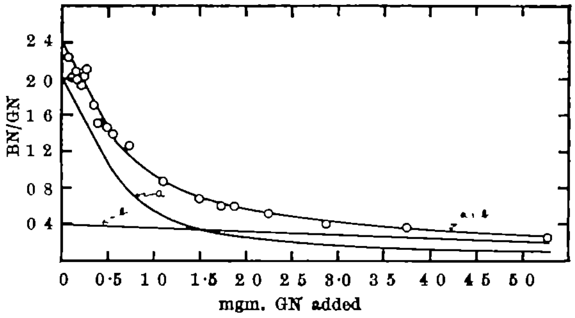

Fig. 3. Quantitative precipitin reaction. *U*2 vs. a hypothetical antiserum containing a mixture of antibodies *a* and *b*. The curves relate the ratios of antibody N to antigen N found in the precipitates individually for *a* and *b* and for the sum of the two ratios. The open circles are the calculated values taken from the experimental results shown in Fig. 1

A quantitative analysis of the cross-precipitin curve would not detect '*C*' in these circumstances, but would reveal whether or not the total number of combining sites for anti-*A* and anti-*B* were different from that of the homologous strain.

This information would be obtained by noting any change in the maximum antibody-antigen ratio. A value of less than 2·4 would indicate fewer identical sites on the related strain, and thereby suggest the possibility of an additional antigen not found on the homologous strain. Alternatively, such data may be interpreted as suggesting a decreased surface-to-volume ratio for the virus particle with a concomitant reduction in the total number of antibody molecules that can be attached to the surface of the virus. An antibody–antigen ratio significantly greater than 2·4 would almost certainly imply a smaller virus particle with identical surface antigens.

Case 2. *A* is present on the test strain, but *B* is not. Cross-absorption would remove approximately one-third of the antibody. The two-thirds remaining, or anti-*B*, would react with the homologous strain.

If the test strain possesses no antigenic grouping other than *A*, the antibody–antigen ratio in the cross-precipitin curve would extrapolate to 2·4. If the test strain possesses other non-related antigenic groups and the size and shape of the virus particle were identical to the homologous strain, then the extrapolated ratio would be less than 2·4, in proportion to the fraction of the total which is *A*.

The absorbed antiserum, which now contains only anti-*B*, will react with only that portion of the surface of the homologous strain which is represented by antigen *B*. Therefore, after cross-absorption the precipitin curve for the homologous reaction should always show a decrease in the maximum antibody–antigen ratio.

Furthermore, if the cross-reactive antibody (anti-*A*) is dissociated and recovered from the heterologous precipitate it, too, when treated with the homologous strain, should reveal a drop in the antibody–antigen ratio. The two ratios, the one obtained with the non-absorbed antibody plus the one obtained with the dissociated antibody, should add up to 2·4.

Case 3. *B* is present on the test strain, but not *A*. The analysis for this case is identical to Case 2.

One further consequence of a multiple system is that partial absorption of the antiserum with its homologous strain should also lead to a fractionation of the antiserum. For example, from Fig. 2 a small amount of antigen nitrogen could be added, sufficient to precipitate all of anti-*A* but only a part of anti-*B*. The remaining unprecipitated antibody would then react in a different way (revealed by a drop in BN/GN ratio) from both the whole unabsorbed antiserum and the antibody recovered from the precipitate after dissociation.

The Single Determinant Case

Two different and detailed analyses of the tobacco mosaic virus/rabbit anti-tobacco mosaic virus precipitin curve, based on the assumption that a single population of antibodies was reacting with one kind of antigenic configuration, have been presented elsewhere[8,9] and so will not be repeated here. For purposes of the present discussion, it is sufficient to note that under the single-determinant hypothesis, none of the preceding attempts to fractionate the antiserum into two discrete and unrelated antibody populations would be successful. Cross-precipitation with related strains would be characterized solely by a drop in titre, since only a portion of the antibody population would be expected to possess any reactivity towards the related antigen. In general, the more perfect the correspondence between the test antigen and the homologous one, the larger the antibody fraction which will react, and the greater will be the similarity in the precipitin curves. No change in the maximum antibody–antigen ratio would be expected either in the cross-reaction (provided again that the surface-to-volume ratio of the heterologous strain is the same as the homologous one), or in the homologous reaction after complete heterologous absorption. Furthermore, no fractionation again into two unrelated antibody populations would be accomplished by partial absorptions with the homologous virus.

A note of caution should be introduced at this point, since such cross-, partial-, or multiple-absorptions have been known to reveal univalent antibody[8]. When present in an antiserum, these univalent antibody molecules commonly co-precipitate with the aggregates initially formed by the divalent antibody and the multivalent antigen. Characteristically, such additions alter the shape of the precipitation curve from a simple quadratic form to one resembling the tobacco mosaic virus/anti-tobacco mosaic virus curves. Thus, the steep initial segment of the antibody curve shown in Fig. 1 may represent essentially the precipitation of divalent antibody, while the more extensive gently rising portion, on the other hand, may indicate the participation of co-precipitating univalent antibody. If this were true, then the precipitation curve observed with antibody dissociated from the homologous partial absorption would be qualitatively quite different from that observed for both the remaining unabsorbed antibody and the original antiserum. This was not observed. The general features of the precipitation curves were always the same as those seen in Fig. 1. Thus, if univalent antibodies were present, they contributed very little to the overall precipitation curve.

It is apparent from the foregoing that the most sensitive datum for distinguishing between the mosaic and single determinant case is the maximum extrapolated value for the antibody–antigen ratio in extreme antibody excess. Any difference in number, or kind of antigenic configuration, will be revealed by some change in the estimation of this maximum ratio. Such changes in ratio were looked for in the tobacco mosaic virus system under investigation, but were not detected.

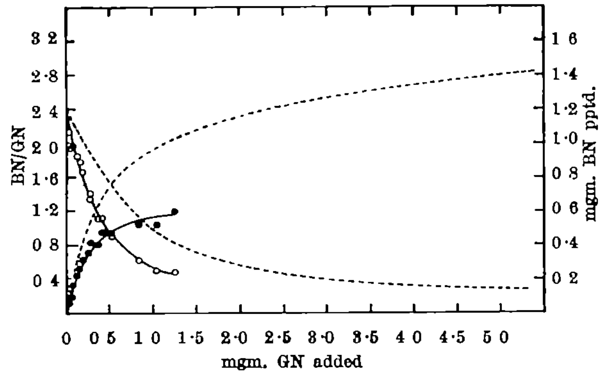

Fig. 4. Quantitative precipitin reaction. *U*1 vs. anti-*U*2 antiserum pool 17–18. Continuous lines are data for the cross-reactive virus *U*1 in antibody excess and corrected to 1·0 ml. undiluted antiserum. Closed circles—antibody N precipitated. Open circles—ratio antibody N to antigen N (BN/GN). Broken lines are the data for the homologous reaction shown in Fig. 1 drawn to the same scale for comparison

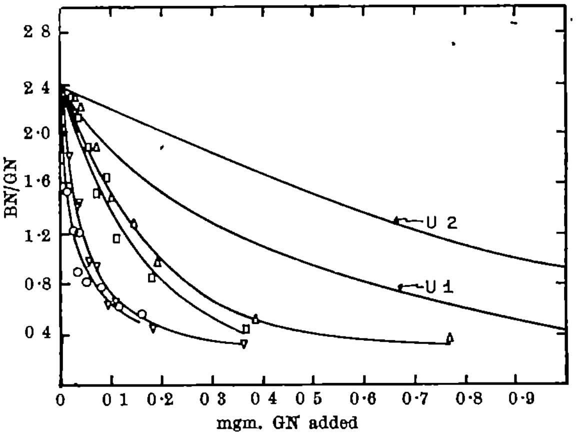

Fig. 5. Quantitative precipitation reaction. Ratios of antibody N to antigen N (BN/GN) revealed after different absorptions of anti-*U*2 antiserum pool 17–18. Lines *U*2 and *U*1 are reference lines of the homologous and heterologous reaction taken from Figs. 1 and 4 and drawn to scale. The following data are uncorrected for the different dilutions encountered: △, after complete heterologous absorption of the antiserum with *U*1 the remaining antibody was precipitated with *U*2; ▽, reaction with homologous *U*2 virus of antibody recovered after acid dissociation from *U*1 anti-*U*2 precipitate; ○, after partial absorption of antiserum with homologous virus, the remaining unabsorbed antibody was treated again with *U*2; □, the precipitate from the partial homologous absorption was dissolved in acid and antibody recovered. This antibody was reacted with *U*2

Fig. 4 relates the quantitative course for the cross-precipitation of *U*1 and anti-*U*2. Apart from the obvious diminution in the quantity of antibody precipitated by the heterologous antigen, the qualitative shape of the precipitin curve is similar to that of the homologous reaction. Furthermore, the extrapolated value for the maximum antibody–antigen ratio does not appear to be different from 2·4. Thus, the antiserum reacts with as many determinant groups on *U*1 as it does on its homologous strain, *U*2.

After complete removal of the cross-reactive antibody by absorption of the *U*2 antiserum with *U*1, the remaining antibody was treated with *U*2. The precipitin curve showed no change in the maximum antibody–antigen ratio. This suggested that no fractionation of the original antiserum, of the kind proposed under the mosaic hypothesis, was accomplished by this procedure. Other attempts to reveal unique antibody populations also failed. The complete data are presented in Fig. 5.

The reciprocal cross-reactions employing an anti-*U*1 antiserum were also investigated. The data were similar to those shown in Fig. 5, suggesting again that only a single antigenic determinant was involved in these reactions. Although this interpretation of the data is the preferred one, at least one other possibility may be entertained.

Tobacco mosaic virus is known from X-ray studies and chemical analyses to be composed of approximately 2,130 protein sub-units[10]. Each of these sub-units is presumed to be antigenic. However, the maximum number of antibody molecules that can combine with an intact tobacco mosaic virus rod has been estimated[9] to be between 700 and 1,200. Thus half or more of the antigenic surface may remain uncombined, as seen in Fig. 6, even under the most favourable conditions of antibody excess simply because of steric hindrance.

Since the experimental evidence tends to favour the notion that only one-third rather than one-half of the sub-units can be combined with antibody, the model described in Fig. 6 will be considered further. Assuming that the model is correct, it is possible to distribute two or three antigenic determinants, but no more, over the surface of the rod in such a fashion that the expected quantitative cross-absorption data would be indistinguishable from those obtained if only a single determinant were present. The different antigenic combining groups must be arranged in the precise serial order of *ABCABCABC* . . . etc., if three are present and *AABAABAAB* . . . etc., if only two are involved. These arrangements are unique. No other, or random distribution of the determinants on the sub-units would yield the same data, nor would four or more different sub-units arranged in any fashion yield comparable results. Consequently, the quantitative precipitation data are capable of distinguishing between the single determinant and the mosaic hypothesis in all cases except the two highly restrictive ones where exactly one-third or two-thirds of the sub-units are *A* and the arrangements are precisely ordered. All the combinational possibilities are described in Fig. 7.

Two general views of the precipitation of tobacco mosaic virus with rabbit antibody have now been presented. In the first and most widely accepted view of precipitation, the tacit assumption is made that in extreme antibody excess all the antigenic sites are combined with antibody. The other, as just described, is that even at best, probably no more than one-third of the antigenic sites are covered. It is under this latter view that one may entertain the notion of a mosaic antigenic structure for tobacco mosaic virus which would be extremely difficult to distinguish from one in which all of the exposed antigenic determinants were identical.

Thus, although the evidence suggesting a single antigenic determinant for tobacco mosaic virus is good, it is for the present not compelling. Positive evidence for the existence of more than one distinct exposed antigen must await a thorough serological analysis of tobacco mosaic virus sub-units of the kind prepared by Ansevin[11] and Wittmann[12]. Until then, the single determinant scheme, with its virtue of simplicity, deserves consideration. It demands the least number of unique antigenic configurations on the viral surface, and a minimum other assumptions as well. The complex results of serological cross-absorptions are charged to the antisera employed. Antibody heterogeneity resulting from immunization with single antigens is commonplace. The problem resolves, therefore, into distinguishing between complexities that stem from an immune response and those inherent in the virus structure.

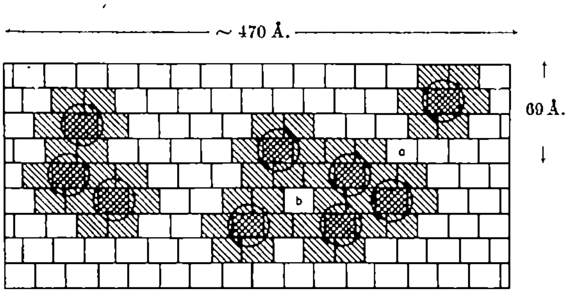

Fig. 6. A diagrammatic representation of a portion of the surface of tobacco mosaic virus showing 3 complete turns of the helix. Each rectangle represents a sub-unit 23 Å. by 29 Å. The circles represent rabbit antibody molecules (with 19 Å. short semi-axis) standing on end attached to an antigenic determinant centrally located on the cross-hatched sub-unit. The shaded areas are those that are blocked from further antibody attachments. In the upper right-hand corner, one antibody molecule exerts its influence over 6 sub-units other than the one to which it is combined. An antibody combining at *a*, will interfere with 2 sub-units not already blocked by the other antibody molecules. An antibody attaching at *b* will interfere with none that is not already blocked. This model permits a maximum combination of one-third of the sub-units with antibody molecules. (Figure taken from Rappaport, I. *J. Immunol*, 82, 526; 1959)

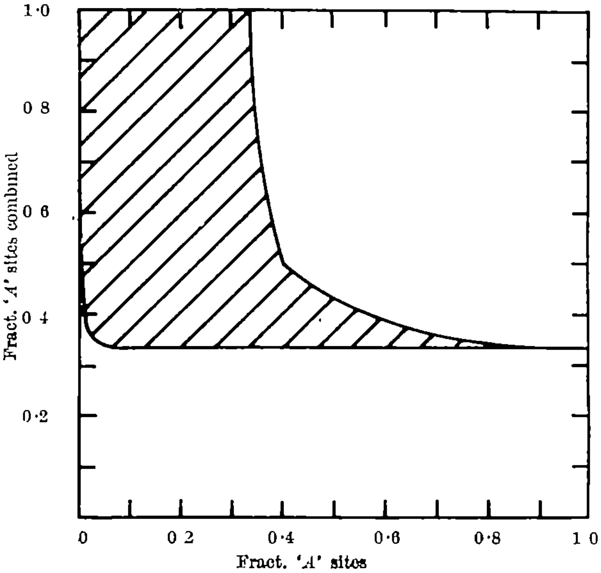

Fig. 7. The relationship between the maximum fraction of *A* sites which can be combined with antibody as a function of the fraction of sites on the tobacco mosaic virus rod which is *A*. This graph is valid for all possible sequences or arrangements of *A* sub-units making up the virus. For example, if the fraction of *A* present were 0 33 and the arrangement were ordered *ABBABBABB* . . . etc., then 100 per cent of the *A* sites could be covered with antibody molecules. If this same number of *A* sub-units were arranged side by side in a block, only 33 per cent could be combined with antibody Any random arrangement of 0 33 *A*'s among the other antigenic sites would result in a maximum combination with antibody somewhere between 33 and 100 per cent, that is, some value in the shaded area. The shaded area, then, indicates the maximum possible combinations for all distributions of *A*

I wish to thank Dr. Sam G. Widman for many suggestions during both the course of the work and the preparation of this manuscript. I also thank Dr. Ray D. Owen for his critical review of the work.

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LETTERS TO THE EDITORS

COSMOLOGY

Cosmological Significance of Angular Measurements of Distant Radio Sources

THE programme of interferometric observations of distant radio sources at present being carried out by H. P. Palmer *et al.* at Jodrell Bank may prove to be of great significance for cosmology. The preliminary results recently published[1] indicate that out of 91 sources 38 were of sufficiently small angular diameter to produce measurable interference fringes at the long base line used (32,000 λ at $\lambda = 1{\cdot}89$ m.). and at least 7 had a diameter between half-power points less than 3 sec. of arc. Furthermore, these 7 were described as having "an extremely high surface brightness which is comparable with that of the intense radio source in Cygnus I.A.U. 19 *N4A*".

In the case of a steady-state universe it has been shown[2,3] that the angular diameters of sources must tend to non-zero lower limits on the observational horizon of the model ($\delta = \infty$). For a Cygnus *A* type source of colliding galaxies, Hoyle has adopted an angular diameter of 80 sec. of arc at the distance of the Cygnus source ($\delta = 0{\cdot}056$) and this leads to a horizon limit of about 4 sec. of arc. The figure 80″ is approximately the angular distance between the centres of the two radio components of the Cygnus source, so that 4″ may be taken to be the limiting half-power diameter of this source if shifted to the horizon. However, the Cygnus source has a ratio of major to minor axes approximately equal to 4, and therefore for sources of this type the average limiting diameter observed east–west would actually be nearer 2½″. It appears, therefore, that the present Jodrell Bank results are not inconsistent with the predictions for a steady-state universe.

In the case of evolutionary universes, provided we can assume that the average intrinsic distance between components of colliding galaxies does not vary significantly with epoch, predictions can be made in special cases. In the Einstein–de Sitter model the average half-power diameter of the Cygnus *A* type source specified above should not fall below a minimum value of 8½″ occurring at $\delta = 1{\cdot}25$, which is more than three times the steady-state limit of 2½″. In a Milne universe the lower limit would occur at $\delta = \infty$ and is twice the steady-state limit, that is, about 5″. In the Dirac cosmology the half-power diameter would fall to a minimum of 13″ at the relatively near point where $\delta = 0{\cdot}73$, and thereafter it would increase. Thus, all these well-known evolutionary models are placed in jeopardy by the present measurements of angular diameter.

Before applying this criterion, it is of course essential to ascertain that the sources are indeed similar to Cygnus *A*, and this can only be done for distant sources by selecting those of a surface brightness comparable to that of Cygnus *A*. According to present ideas only colliding galaxies could generate an energy flux of this high order.

In this connexion, however, attention is directed to a fact that does not appear to have been realized before, namely, that the surface brightness to be inferred for extra galactic radio sources depends strongly and explicitly on their red shift, and therefore cannot be calculated accurately from the angular diameter and apparent intensity alone, except when δ is small. Since the interpretation of any observational test in cosmology involving radio sources depends critically on what is assumed to be their luminosity function, this is evidently a matter that requires careful attention at the present time.

The formula for surface brightness may be derived as follows.

In the usual notation the general cosmological metric of homogeneous isotropic expanding world models is :

$$ds^2 = c^2dt^2 - R^2(t)\,\frac{(dr^2 + r^2d\theta^2 + r^2\sin^2\theta d\varphi^2)}{(1 + kr^2/4)^2} \qquad (1)$$

Consider a source the permanent (co-moving) spatial co-ordinates of which are (r,θ,φ). Let the surface brightness distribution function of a given element of its surface area, at the emission frequency ν and at the epoch of emission t, be $B(\nu,t)$ (W. m.$^{-2}$ (c./s.)$^{-1}$ ster.$^{-1}$). Hence the energy radiated from a surface element of proper area ΔA in the interval $t, t + dt$, in frequency-range $\nu, \nu + d\nu$, and in solid angle $d\omega$ will be :

$$B(\nu,t)d\nu dt d\omega \quad \Delta A \qquad (2)$$

Then, if $d\omega$ corresponds to 1 sq. m. at the observer who is located at $r = 0$ at the epoch of reception t_0, we know that[4] :

$$d\omega = 1/\{R^2(t_0)r^2/(1 + kr^2/4)^2\} \qquad (3)$$

Also, if the frequency-range of reception resulting from red shift is $\nu_0, \nu_0 + d\nu_0$, and if $\Delta I(\nu_0)d\nu_0$ is the rate at which energy from ΔA in this frequency-range falls on 1 sq. m. at the observer, we may express the degradation of the energy (2) due to red shift in the form :

$$\Delta I(\nu_0)d\nu_0 dt_0 = \{B(\nu,t)d\nu dt d\omega \;\Delta A\}/(1 + \delta) \qquad (4)$$

where δ is the red shift of the source and :

$$\frac{\nu}{\nu_0} = \frac{d\nu}{d\nu_0} = \frac{R(t_0)}{R(t)} = \frac{dt_0}{dt} = 1 + \delta \qquad (5)$$

Furthermore[3] :

$$\Delta A = \frac{R^2(t)r^2 \;\Delta\Omega}{(1 + kr^2/4)^2} \qquad (6)$$

where $\Delta\Omega$ is the solid angle subtended by the area ΔA at the observer. Whence, by (3)–(6) :

$$\Delta I(\nu_0) = \frac{B(\nu,t)\;\Delta\Omega}{(1 + \delta)^3} \qquad (7)$$

and so the required surface brightness function is :

$$B(\nu,t) = \frac{\Delta I(\nu_0)}{\Delta\Omega} \cdot (1 + \delta)^3 \qquad (8)$$

We note that this formula applies to either an evolutionary model or a steady-state model. In the steady-state case $B(\nu,t)$ would be statistically independent of t, unless a selective bias depending on distance were operating in the observations.

On the other hand, the formula that appears to be generally adopted by radio astronomers has been given[5,6,3], and evidently followed in the published calculations even for very distant sources, as :

$$B(\nu_e) = \Delta I(\nu_0)/\Delta\Omega \tag{9}$$

Since this formula is valid only for a Newtonian and non-expanding universe it may lead to serious error when applied to distant sources in the actual universe. For example, in the steady-state universe the received intensity $\Delta I(\nu_0)$ at a given frequency ν_0 must tend to zero when the source approaches the observational horizon of the model ($\delta \to \infty$). But, as I have remarked earlier, $\Delta\Omega$ tends to a non-zero limit for a given ΔA. Consequently, if in such a universe B were calculated by (9), instead of (8), it would be found to tend to zero. This is evidently a contradiction of the postulated steady state.

Another important point to notice is that $B(\nu,t)$ is calculable in this way for the emission frequency only, and in general at the epoch of emission only. Given $B(\nu,t)$, the local surface temperature would be calculable from the Rayleigh–Jeans law in the usual way, for the frequency ν and epoch t.

I have applied the formula (8) to calculate the surface brightness of the recently identified source I.A.U. 14*N*5*A*, the red shift[7,10] of which is 0·461 and the half-power diameter[1] is 4½″. Adopting a half-power apparent intensity of 37×10^{-26} W.m.$^{-2}$ (c./s.)$^{-1}$ at 159 Mc./s. for this source one finds an average temperature at the emission frequency of 232 Mc./s. equal to $1{\cdot}86 \times 10^{8}$ deg. K. On the other hand, the Cygnus *A* source, assuming it has a principal area of radiation of $2 \times 50'' \times 30''$ as given by Jennison and Das Gupta[8], has an average surface temperature of 7×10^{7} deg. K. at 232 Mc./s. Consequently, the 14*N*5*A* source was more than 2½ times as bright as Cygnus *A* at this frequency at their respective epochs of emission. This ratio would evidently hold over the wide range of frequencies for which the spectral indices of these two sources are nearly equal[9]. The ratio may even be as much as twice this value since we have not allowed for possible separation of components in 14*N*5*A* as we have done for the Cygnus *A* source.

It is indicated that the luminosity distribution of radio sources may be wider than has been anticipated. On the other hand, if it were found that the average brightness of the sources increased systematically with distance, assuming there was no selection effect, then this would be evidence of an evolutionary universe.

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The Interpretation of Cosmology

WE are submitting herewith some further reflexions on the communication by Prof. W. H. McCrea[1], which dealt with uncertainty in cosmology.

(1) The inaccuracy of observations necessarily increases with distance. However, it cannot be excluded *a priori* that in the future the observational error concerning any chosen distant part of space could be brought below any pre-determined limit.

(2) The present cosmological theories imply a fairly large number of definite statements on future observations. This is implicitly admitted by McCrea, who points out with considerable generality that they differ from each other by $(1 + z)$, that is, by a well-determined factor[2] (z is the red shift).

It obviously follows from the above that it is a mistake to exclude the possibility of discrimination between the existing theories. (Discrimination does not mean, of course, verification.)

(3) According to McCrea, predictions relating to the immediate future of remote points of the "world picture"[3] imply an unavoidable uncertainty increasing with $(1 + z)$, that is, with the same factor by which the predictions of the existing theories differ from each other. From this he infers that the theories mentioned above cannot be distinguished from each other.

We wish to point out that in (1) the word 'prediction' is used in two different senses :

(*a*) Prediction for the immediate future of points of the world picture.

(*b*) Prediction of the results of measurements not yet carried out but considered as being realizable at an epoch, contemporary in a cosmological sense.

It is known only in theories based on an adequate cosmological principle that the two concepts are connected : in the case of these theories the immediate future of a point is definite (it is identical to the presence of a well-determined neighbouring point in the world picture). It seems to us that to go over from one meaning to the other within one and the same train of ideas may at best be justified by accepting the definite immediate future.

After these preliminaries let us examine one of the most important inferences of Prof. McCrea's communication, according to which it is in principle impossible to make a distinction between the two basic types of cosmological models. As mentioned above, if we accept our reflexion (1), we cannot deny the possibility of making a decision between the current theories. Therefore the only way to repudiate this possibility is to disprove (1) ; that is to say, to prove an irreducible uncertainty in observation. Instead of this, McCrea asserts that an irreducible uncertainty subsists as regards predictions. This, in his opinion, would characterize theories which are correct in the sense of not supposing homogeneity. It is evident that this can by no means serve as a basis for inferences regarding the distinction between existing homogeneous models. Not only is the logically relevant premise substituted by an inadequate one, but also in the same syllogism no distinction is made between the two kinds of predictions and thus McCrea's wording involves the negation of his own premise, that is, the assumption of the definite immediate future. The inaccuracy of the conclusion can be sufficiently proved by a single example : although the predictions of the Einstein universe differ from the evolutionary models only by a similar function of some distance parameter yet observations have disproved them, beyond any doubt.

These objections in themselves do not affect McCrea's main idea, according to which, independently of the degree of theoretical development and instrumental improvement, full information on the effects influencing some remote part of the universe may be obtained only by observations continued throughout an objectively determined and cosmologically considerable period of time. McCrea infers from this that only theories embracing finite parts of space-time may be considered as realistic.

In our opinion the most valuable idea in McCrea's communication is the delimitation of what in principle is obtainable by a newly developed investigation programme. Its main deficiency is that he wants to insert the uncertainty, discovered in his own "cosmology", into the framework of well-defined theories of present cosmologies.

Neither can we accept McCrea's scientific-philosophical point of view, as we do not consider the cosmological investigations built up on working hypotheses which cannot be established directly to be superfluous. The possibility of model examinations might be understood from the following: in the first place, Nature is entitled to reject scientific prejudices (speculative considerations which are essentially subjective must be pushed into the background). Here, as in all other scientific examinations, we have to take it for granted that any inaccurate statement concerning Nature must, sooner or later, clash with an observational fact. Consequently, every logical theory built on scientific basis is justifiable, provided it is "vulnerable"[4], for example, in its predictions, so that there is the possibility for Nature to prove that it is wrong. No examples are needed to demonstrate the tremendous progress which could be made in science, through the discussion of some theories which seem, for the moment, to be controllable only in indirect ways, even if their formal aim is the examination of the universe as a whole, and the decision as to their scope is postponed for the future. Therefore we consider model investigations as one of the possible theoretical methods of cosmological examination available at the present.

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ASTROPHYSICS

Solar Wind and the Gegenschein

To account for the motion of comet tails, Biermann[1] has suggested that there is neutral ionized hydrogen streaming outward from the Sun, and that at the Earth's orbit this plasma has a density typically of the order of 10^2 protons cm.$^{-3}$ and a velocity of approximately 500 km. sec.$^{-1}$. During periods of violent solar activity the velocity increases possibly to 1,500 km. sec.$^{-1}$ and the density increases by one or two orders of magnitude. Parker[2] has also shown that for theoretical reasons there is a hydrodynamic expansion of the solar corona, referred to as the 'solar wind', and that at the Earth's orbit the solar wind has approximately the same density and velocity as estimated by Biermann.

The interaction of a solar corpuscular emanation with the geomagnetic field is exceedingly complex, and for many years it has been invoked as the basic mechanism for explaining a wide variety of geomagnetic, auroral, and upper atmospheric phenomena. Yet apart from the deflexion of comet tails there is singularly little direct observational evidence in support of the solar-wind hypothesis. The purpose of this communication is to direct attention to some additional observational evidence which supports the idea that there is an interplanetary plasma streaming away from the Sun with a velocity close to that estimated by Biermann and Parker.

Since the pioneer work of Chapman and Ferraro[3], it has been agreed generally that the effect of a solar wind impacting against the geomagnetic dipole field is to compress the field into a radius, variously estimated by different authors, of 5–15 Earth radii on the Sun's side of the Earth. Until recently, there was rather less agreement on the subject of what happens to the geomagnetic field on the night-side of the Earth. It now seems with reasonable certainty that some of the magnetic flux from the polar regions, above the auroral latitudes, is carried round to the night-side and the flux-lines are stretched away from the Earth by the solar wind[4]; this 'arch' of polar magnetic flux does not rotate with the Earth or with the magnetic field at lower latitudes. Some of the flux-lines originating from the geomagnetic polar regions are entangled and embedded in the solar wind[5], and are carried away[6] as a "wind carries smoke from a chimney stack", and thus form[7] a 'magnetic tail' to the Earth. Piddington[7] has also made the interesting suggestion that the gegenschein is located within the Earth's magnetic tail.

The gegenschein, or counterglow, is a faint patch of light in the night sky close to the ecliptic and directly opposite the Sun. Visual[8,9], photometric[9–11] and spectroscopic[9,12] observations by the Russian workers Astapovitch, Divari, Fesenkov, Karimov and Rozhkovski have established that the gegenschein glow is elliptical in shape and has a size roughly of 10° by 6°, with the major axis lying nearly along the ecliptic; that it has short- and long-term fluctuations in size and brightness which apparently have some correlation with solar, auroral and airglow activity; that its position as determined by parallax measurements is variable and is at a mean distance of 20 Earth radii; that its emission spectrum in the visible range consists principally of lines common to the night airglow; and that it has a small but variable displacement to the west of the midnight meridian amounting to a mean value of 3°. Roach and Rees[13] have made photometric studies, and conclude that the gegenschein and the adjacent zodiacal light are due to separate and distinct concentrations of particles. It thus appears that the known properties of the gegenschein are consistent with the idea of a slightly luminous magnetic tail to the Earth, and that the magnetic tail is in fact also the gaseous tail proposed by Arrhenius, Houzeau[14] in 1888, and later discussed by Evershed[15] in 1902. The luminosity is presumably owing to the presence of atmospheric-type atoms which, initially, have migrated along the polar flux-lines as ions and become enmeshed in the magnetic tail.

Since the Earth has an orbital velocity of $v_e = 30$ km. sec.$^{-1}$, it is immediately obvious that a solar wind of velocity v_r streaming radially away from the Sun will form a magnetic tail which is directed to the west of the midnight meridian, and if the angular displacement is α, then:

$$v_r = v_e \cot \alpha \qquad (1)$$

Using the observed value of $\alpha = 3°$ it follows that the solar wind has a mean velocity of 600 km. sec.$^{-1}$. This velocity is remarkably close to the value estimated by Biermann and Parker.

On looking for alternative possible causes of the gegenschein displacement one notices that if neutral atmospheric molecules are ejected, by some unknown means, from the Earth at a velocity v in a direction away from the Sun, the conservation of angular momentum gives a westward displacement of:

$$\alpha = v_e r / vR \qquad (2)$$

for $r \ll R$, where R is the radius of the Earth's orbit and r is the distance of the molecules. For r equal to 20 Earth radii, and v equal to 1 km. sec.$^{-1}$ corresponding roughly to the mean speed at a temperature of 10^3 °K., one finds that α is 3° as required. However, it would seem that the main weaknesses with this explanation are: first, that α is proportional to r, and therefore a gaseous tail observed from the Earth would tend to spread westward in a noticeably asymmetric way; secondly, that a large distribution in the values of v would mar the possibility of a definite and confined region of luminosity; and thirdly, it encounters the hitherto unsolved problem of the gaseous tail theories of accounting for a practically non-divergent jet of gas[16].

In all probability the solar wind at the Earth's orbit is not directed entirely radially from the Sun, and therefore equation (1) must be modified to:

$$v_r = (v_e - v_\perp) \cot \alpha \qquad (3)$$

where $v_\perp$ is the transverse velocity of the solar wind at the Earth's orbit in the direction of the Earth's orbital motion. If the angular momentum of the solar wind is conserved from the inner corona, the transverse velocity at the Earth is 0·01 km. sec.$^{-1}$ and is too small to affect noticeably the position of the gegenschein. But if the solar wind and the Sun are electromagnetically coupled[17] and tend to rotate together, the maximum possible distance from the Sun for co-rotation is 0·17 of the radius of the Earth's orbit, and corresponds to the Kepler orbit in which the angular velocity is that of the Sun. In this case the transverse velocity at the Earth is 12 km. sec.$^{-1}$, and according to equation (3) the solar wind has therefore a radial velocity of 360 km. sec.$^{-1}$ for a displacement of 3°.

It must be admitted that our present knowledge of the gegenschein is inadequate and is far from being in a satisfactory state. The little that is known, however, is not inconsistent with the idea of a solar wind interacting with the geomagnetic field and producing a slightly luminous gas-magnetic tail to the Earth. Therefore, if it is accepted that (1) the solar wind carries away a magnetic tail from the Earth, and that (2) the gegenschein is a luminosity associated with the magnetic tail, then the characteristic westward displacement of 3° confirms that the solar wind has a mean velocity close to 500 km. sec.$^{-1}$.

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Existence of Net Electric Charges on Stars

PROF. V. A. BAILEY has recently proposed[1] an equation relating the electric charge on a star to the Newtonian gravitational constant and to the mass of the star. This equation has the form:

$$Q = \beta G^{1/2} M \qquad (1)$$

where Q is the electrostatic charge, β is a dimensionless constant of approximately 0·03 for the Sun, G is the gravitational constant, and M is the mass of the star. For the Sun, Q turns out to be $1{\cdot}5 \times 10^{28}$ e.s.u. I wish to point out that equation (1) is closely related to a similar equation which I have obtained for the gravitational fields in the interior of fundamental particles (electrons and protons) by applying the Weyl theory of gauge invariance to the Einstein–Ricci curvature tensor.

Since in the original Weyl theory the dimensions of a charged particle change as it moves from point to point in an electromagnetic field, it is necessary to alter this theory by imposing two constraints if it is to lead to results with physical meaning[2]. The first of these constraints limits the motion of the particle to that path between any two points along which the total change in the dimensions of the particle is a minimum. This at once leads to the Lorentz pondermotive force. The second constraint limits the possible paths still further by requiring that the dimensions of the particle take on their initial values when the particle returns to its starting point. This gives us the Bohr–Sommerfeld quantum condition.

If one[3] now enlarges the Einstein–Ricci curvature tensor by imposing the condition that it be gauge invariant, the additional terms that are obtained are related to the electromagnetic 4-vector and Maxwellian field. If an invariant is constructed with this enlarged tensor, and this is then taken as the Lagrangian density, one obtains the second-order Dirac equation as written down by Feynman and Gell-Mann[4] provided the radius of Gaussian curvature in the neighbourhood of the electron is placed equal to the Compton wave-length $\hbar/mc$.

Since the formula for the Gaussian curvature contains the gravitational constant G as a factor, one is thus led to the following equation for G inside an electron (or proton):

$$G = \hbar c/m^2 = \alpha^{-1}(e/m)^2 \tag{2}$$

where α is the fine structure constant, $e^2/\hbar c$, and e is the electronic charge. Thus the value of the gravitational constant inside an electron is of the order of 10^{34} C.G.S. units.

If we take the square root of (2) we obtain Bailey's equation for the Sun:

$$G^{1/2} = \alpha^{-1/2}(e/m) \tag{3}$$

provided we identify his constant β with the square root of the fine structure constant, $e/(\hbar c)^{1/2}$. Of course, the two quantities are numerically different, but that is simply because the quantity that we must use in place of $e/(\ c)^{1/2}$ if we wish to apply (3) to the Sun is:

$$\beta = Q/(2I\omega c)^{1/2} \tag{4}$$

where I is the moment of inertia of the Sun, ω is its angular velocity, and c is the speed of light.

In other words, what is significant in formula (3) is not the appearance of Planck's constant but rather the appearance of an angular momentum. If we are to apply (3) to the Sun, we must introduce a kind of 'fine structure constant' corresponding to the angular momentum and charge of the Sun.

If in (4) we introduce Bailey's value for Q and use the value of I obtained from calculations of radius of gyration for solar models[5], the value of β is about 0·03 as postulated by Bailey.

What is perhaps more interesting is that (2) leads to the expression relating the mass and the radius of a finite universe as first derived by Einstein in 1916. If we consider the universe as a closed system with intrinsic angular momentum $I\omega$, then the radius R of the universe must be such that $R\omega = c$ or $\omega = c/R$. If we now take I to be kMR^2, where k is a numerical factor approximately unity, then the expression for G for the universe as a whole becomes:

$$G \simeq I\omega c M^{-2} = kRc^2M^{-1} \tag{5}$$

This is just Einstein's formula for a universe of radius R and mass M. It is interesting to note that an equation of the form (2) has been suggested by Chandrasekhar[6] as the most general combination of the constants $\hbar$, c and G, and the mass of the proton.

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PROF. MOTZ'S model of an electrically charged fundamental particle is of particular interest because it directly contradicts the commonly held view that gravitational fields on the atomic scale are quite negligible. By means of some bold but simple hypotheses he appears to obtain a model of an electron (or proton) which is capable of holding its parts together against the electric forces of repulsion between these parts.

Prof. Motz's comparison of his equation (2) with my equation (1) is also of interest, because it extends the similarity between astronomical systems and atomic systems which was first demonstrated by Bohr and Sommerfeld's theories and their extension by Goudsmit and Uhlenbeck; but here the similarity is one between single constituents of these two kinds of systems.

It is remarkable that the order of magnitude, 0·03 for the pure number β in the equation (1) is consistent with the values derived by Motz from his table of solar models published in his ref. 5. It should, however, be noted here that on reconsideration of the data on which I based my first estimate of β, I have recently reduced its value to about 0·006 and correspondingly reduced the Sun's charge to about -3×10^{27} e.s.u. But the uncertainties in the data make even this recent estimate merely one of order of magnitude.

In a recent communication[1] on the apparent steady component of the interplanetary magnetic field, it is shown that the findings of the magnetometer on the space probe *Pioneer V* during the first 50 days of operation strongly support the hypothesis that the Sun carries a large negative charge. But from that communication it may be inferred that in order to determine the value of β experimentally in this way it would be necessary to make measurements by means of instruments carried in space probes moving in different orbits. Such a determination of β is highly desirable, since it would be not only of interest to astronomers but also might be of value in connexion with Prof. Motz's considerations.

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PHYSICS

Effect of Dissolved Air on the Viscosity of Water

WATER at 20° C. is now widely used as the primary standard for viscosity measurement[1], the viscosity being taken to be 0·01002 poise as obtained by Swindells, Coe and Godfrey[2], and confirmed by Roscoe and Bainbridge[3]. The International Organization for Standardization has recently proposed the international adoption of this value, but the question has arisen whether the viscosity depends significantly on the presence of dissolved air in the water[4]. We have now made some very accurate measurements to determine the effect of dissolved air, and found it to be insignificant for present standards requirements.

The viscosity of air-saturated water has been measured relative to that of air-free water. The measurements were made using sealed suspended-level viscometers incorporating fine platinum contacts by means of which electronic timing equipment is triggered. The viscometers were immersed in a stirred water-bath, the temperature of which was kept constant to within ± 0·0007 deg. C. by a proportioning controller and measured with a platinum resistance thermometer. Within a set, the concordance of flow-times was 1 part in 50,000.

The ratio of the flow-times obtained at 20° C. with and without air at atmospheric pressure in the viscometers was found to be 1·00125 ± 0·00009 at a 95 per cent confidence-level. To obtain the corresponding ratio of kinematic viscosities, this figure must be reduced by 4 parts in 100,000 for the effect of the pressure difference on the dimensions of the viscometers, and by a factor of 1·00119 for the effect of the buoyancy of the water with respect to the water-saturated air. The resulting viscosity ratio does not differ significantly from unity. More specifically, the effect of air at atmospheric pressure on the kinematic viscosity of water does not exceed 13 parts in 100,000 at the 95 per cent confidence-level. This statement relates to the combined effects of the dissolved air and the pressure; but it can be shown independently, by extrapolating data available for higher pressures, that the direct effect of a pressure of one atmosphere is to change the viscosity by no more than a few parts in 100,000.

Likewise, at 25° C. and 30° C. no significant effect was found.

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A Phenomenon in *n*-Hexane prior to its Electric Breakdown

A PRELIMINARY photographic study has been made using a schlieren optical system in which a 1-microsecond flash-light source was arranged to flash five times in synchronism with a single-step function voltage applied to electrodes immersed in highly purified *n*-hexane. The events were recorded with a rotating-mirror camera.

In the examples shown in Fig. 1, the first photograph in each series shows the undisturbed state of the liquid prior to the application of the voltage. Each second photograph, taken 7 μsec. after the application of the voltage, shows that the refractive index in a localized region at the pointed cathode has become different from that in the surrounding liquid. The extent of the localized region was observed to be greater with higher voltages under otherwise similar conditions.

The phenomenon at the cathode can disappear (Fig. 1*A*) without breakdown ensuing, even with the voltage maintained. On the other hand, Fig. 1*B* shows a spark breakdown, which is self-luminous and therefore recorded, about 20 μsec. after the application of the voltage. The later photographs record the bubble originating from the spark. Fig. 1*C* shows similar phenomena in a point-plane gap with the breakdown about 10 μsec. after the application of the voltage.

All these phenomena are detectable with a shadowgraph technique, which is less sensitive than the schlieren technique; thus the refractive index of the region at the cathode must be greatly different from that of the surrounding liquid, but it is not known whether it is higher or lower.

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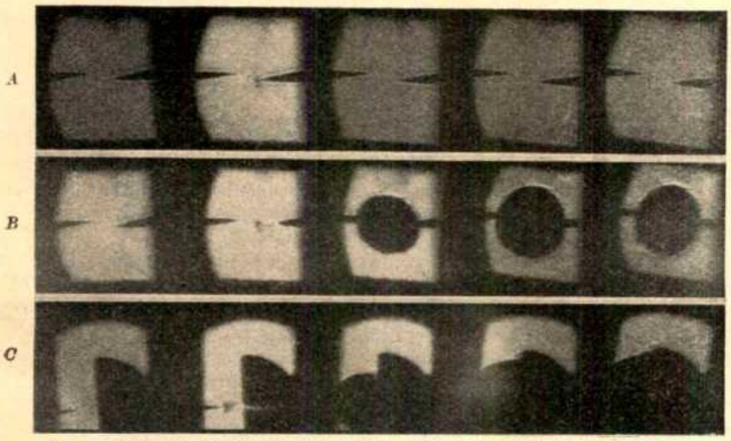

Fig. 1. Synchronized schlieren photographs of pre- and post-breakdown phenomena in *n*-hexane. In each series the first photograph was taken 70 μsec. before the application of a step function voltage, and the remaining photographs were taken, respectively, 7, 80, 150 and 220 μsec. after the application of the voltage. Ratio voltage/gap-length: *A*, 325 kV./cm.; *B*, 335 kV./cm.; *C*, 330 kV./cm.

Emission of Soft Electromagnetic Radiation from Metals by the Impact of High-Energy Protons and Positive Ions

IT is known that the impact of fast protons[1] and α-particles[2] on metals results in the emission of their characteristic X-radiation. It has not been reported, however, whether ultra-violet and visible radiation are also emitted from metals when high-energy protons and positive ions strike them. Sir J. J. Thomson[3] predicted as early as 1914 that the impact of positive ions on metals may result in the emission of soft electromagnetic radiations. Evidence supporting this view has been given in recent experiments, where it is shown that ultra-violet and visible radiation is emitted when positive ions and protons of moderate energies strike a nickel surface[4]. These experiments have now been extended to protons and positive ions of energies of 400–700 keV.

A proton beam was obtained from an Oliphant ion source[5] of our 1·2-MeV. Cockcroft–Walton generator, supplied by Messrs. Philips Electrical Co. of Eindhoven, Holland, and described earlier by one of us[6]. It was analysed with a 15° permanent magnetic analyser using an adjustable magnetic shunt. The voltage for the ion source was obtained from a 0–50-keV. transformer energized by a remote controlled 0–220-V. a.c. generator driven by a large insulating belt, both installed in the top of the accelerator along with the Oliphant discharge tube and the gas reservoir.

The output voltage of the transformer was rectified with a Philips high-tension five-ring mercury rectifier valve and was stabilized with an R.C. filter, and the gas-flow to the ion source was regulated by a remote-controlled needle valve. The discharge was run at 10–20 m.amp. with discharge potential of 20–30 kV. Protons travelled through a long metal tube cooled with liquid air and entered the target chamber through a narrow aperture before striking the target 10 cm. below. The main accelerating column was exhausted with a large diffusion pump backed by a Kinney pump. A separate set of these pumps evacuated the proton-collimating tube and the target chamber through a large liquid-air metal trap continuously during experiments.

Observations were taken with proton current varying from $1 \cdot 0 \times 10^{-7}$ amp. to $4 \cdot 0 \times 10^{-6}$ amp. Radiation emitted from the target was detected with a photoelectron E.M.I. multiplier type 6260 and with associated electronic equipment, and was studied with Kodak gelatine filters supplied by Kodak, Ltd., of London.

It was observed that soft electromagnetic radiation in the visible and ultra-violet regions is emitted from a nickel target, in a well-degassed as well as in an un-degassed condition. The intensity of the radiation in the ultra-violet region above 3300 Å. was greater than that of the visible part of the spectrum in either case. The total intensity of the radiation of wave-length above 3300 Å. rises more rapidly than the increase in the proton current and is always greater from a dirty surface than from a clean one. It shows, however, a decrease of about 20 per cent as the energy of the protons is raised from 400 to 700 keV. at a constant proton current. The spectrum in the visible region was continuous, with a pronounced flat peak between 4600 and 4900 Å. Radiation of wave-length above 5200 Å. was not measured, however, on account of experimental limitations. A spot visible to the naked eye appears on the target with as small a current as $1 \cdot 0 \times 10^{-7}$ amp.

In one set of experiments the target was well degassed under high vacuum by electron bombardment at bright red heat for several hours before observations were taken. The degassed target was left under vacuum overnight and observations were repeated the following day after degassing it again, when the results were found to be reproducible.

Experiments have also been performed with unresolved hydrogen ions obtained from a 30-Mc./sec. radio-frequency source recently installed in our accelerator. Hydrogen flowed from the gas reservoir to the radio-frequency discharge tube through a remote-controlled nickel valve the temperature of which determined the gas-flow and thus the discharge pressure. The voltages for the focusing lens and the ion-extracting probe were obtained from 50-kV. and 5-kV. selenium rectifier Cockcroft–Walton cascade sets, respectively, which were both remote controlled and were placed in the top of the accelerator with the power supplies for the 30-Mc./sec. oscillator. The positive ion current to the target was adjusted by varying the pressure of the discharge gas or the voltage of ion-extracting probe or both and was varied from 4×10^{-6} amp. to $15 \cdot 0 \times 10^{-6}$ amp. while the energy of the ions was kept at about 400 keV. We have observed that the radiation emitted from the nickel target, both from its dirty and clean surfaces, gives a continuous spectrum in the visible region in this case also. The spectral intensity distribution curve is flat from 4000 to 4700 Å., beyond which it falls rapidly. The intensity of the visible radiation with current of 10×10^{-6} amp. and above was too high for direct measurement, and Kodak neutral density filters were used to cut it down for recording with the electronic circuits. In one set of observations the ion current was raised to a value high enough to make the target glow bright red due to bombardment when it was kept under this condition for several minutes for degassing, and was then suddenly decreased to about 4 μamp. for taking observations from a clean surface freshly prepared.

We have checked that the radiation detected by us is not due to the secondary electrons emitted from the edges of the aperture by the protons entering the target chamber, nor is it due to their heating effect on the nickel target. Details of the experiments will be published elsewhere.

We are indebted to Sir John Cockcroft and to Sir Mark Oliphant for advice and encouragement, and to Dr. Paul W. McDaniel, director of the Research Division, U.S. Atomic Energy Commission, for helpful discussion and suggestions. We also thank the U.K. Government for the gift of high-tension selenium rectifiers and the 30 Mc./sec. radio-frequency ion source recently installed in our accelerator under the Colombo Plan.

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Flow Patterns in Particle Beds

THE technique of observing gas-flow patterns in fluidized beds described in a recent communication[1] is a useful contribution to the methods available for investigating fluidization behaviour. A comparable technique has been developed here using dye injection for tracing the flow pattern of water passing through particle beds. Though this technique was found useful in the pre-expansion stage, it was the reverse during bed expansion when it actually tended to mask the interesting phenomena shown in Fig. 1. These photographs were taken by transmitted light without the use of dyes, and show the activation of a bed of 100μ glass spheres by water. Local bed expansion or increased voidage is indicated by increase in transmitted light. Photograph *C*13 shows the approximate steady-state conditions in a bed which, viewed superficially, has all the appearance of uniform and smooth particulate fluidization. The series *A*1–*A*12 (of which *A*1, 3, 6 and 12 are reproduced) shows the development of a flow pattern in the same bed at a much lower superficial water velocity. The last of that series again approximates to steady-state conditions, the only changes thereafter being a slow meandering of the flow channels. Not seen in the photographs but observed visually are occasional series of mushroom-shaped low-density discontinuities rising in one or more of the channels.

The visual study of particle bed activation by air was studied here in a parallel-plate apparatus using

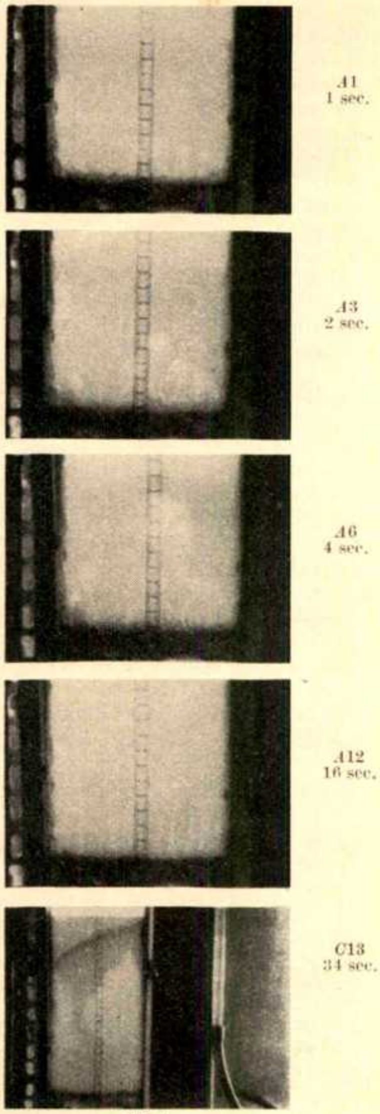

Fig. 1

mixtures of particles with differently coloured size ranges. It was possible to observe the circulation of 300-mesh particles of alumina dyed red, within air bubbles held stationary by stiction effects in a bed of 100 mesh undyed alumina. A modification of this apparatus, suitable for water fluidization, was made with the prime object of investigating what appeared to be anomalous and hitherto unreported behaviour, namely, an apparent bubbling stage in water fluidized beds of small, moderately dense particles. In all cases studied, the expansion from fixed bed to particulate fluidization passed through a stage closely resembling the gentle bubbling stage in air fluidization. Streams of mushroom-shaped pseudo-bubbles can be observed rising in narrow channels within water-activated beds of alumina and glass spheres within the size range of 60–200μ, but it is not clear whether these are of the same nature as bubbles in air-activated beds, or some type of vortex phenomenon. In the case of glass spheres larger than 1 mm. diameter, horizontal striation can be observed with low-density strata being propagated upwards continuously and evanescing at the top of the bed. These occasionally break and re-form, giving an appearance of mushroom-shaped bubbles.

The term 'parvoid' is suggested as a generic term to describe these and other low-density discontinuities in fluid-activated beds. With 2-mm. glass spheres, parvoids of mushroom shape ranging from 1 cm. to 5 cm. in width can be seen rising at some 5 cm. per sec. through the dense bed at superficial water velocities of some 2 cm. per sec. These parvoids give the impression of being completely void of particles, in contrast to parvoids in beds of smaller particles.

The possible existence of discontinuities and non-uniformity in what have hitherto been considered to be homogeneous beds may have some relevance to much published work in the literature. A complete account of these discontinuities with a tentative theory as to their formation and relationship to fluidization behaviour will be published elsewhere. There is evidence that what is known as particulate and aggregative fluidization may be occurring at one and the same time in a fluidized system, and that it is the proportion in which they occur that defines the one type of fluidization as against the other. If this is so, one could expect a transition from one to the other in any fluid-activated particle system, as the fluid velocity is increased in stages.

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BIOPHYSICS

Molecular Sieve Behaviour of Dental Enamel

NORMAL mammalian tooth enamel is highly calcified, containing 97 per cent mineral and 3 per cent organic material and water. The mineral is hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, and it is associated with a protein constituent of the keratin type[1]. From histological studies it is well known that normal enamel is made up of structural units which are prismatic in shape and contain mainly crystallites of hydroxyapatite. The organic constituent is concentrated at the margins of these prisms but it is also present in smaller amounts within them. In the prisms the crystallites are highly oriented, with their optic (fibre) axes approximately parallel with the longitudinal axes of the prisms. This submicroscopic structure of normal enamel is shown diagrammatically in Fig. 1. The fully formed enamel can be broken down either *in vitro* by organic acids such as lactic acid, or *in vivo* by the processes concerned in carious decay. In each case the mineral is gradually dissolved out until, eventually, the structure of the enamel is destroyed[2].

Since hydroxyapatite is a uniaxial mineral possessing negative birefringence, normal enamel shows a negative intrinsic birefringence relative to the longitudinal axis of the prism due to this inorganic component. This is the only birefringence shown by normal, fully formed enamel. However, during both formation and acid breakdown of normal enamel, it has been shown that a considerable number of freely accessible spaces exist between the crystallites in the prisms, and these spaces can give rise

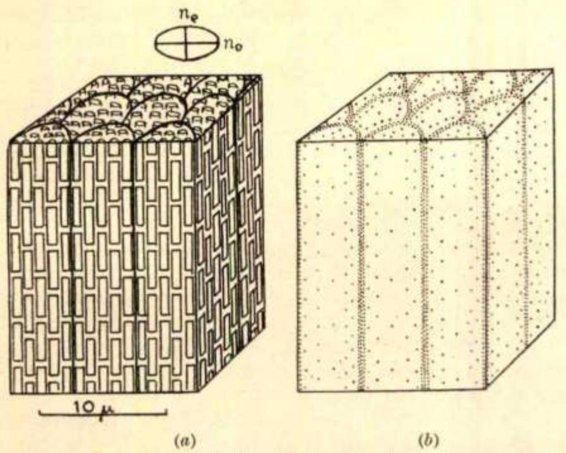

Fig. 1. Diagrams illustrating the fine structure of normal tooth enamel. (a) The arrangement of mineral elements. The enamel consists of columnar prisms within which are tightly packed, rod-shaped crystallites of hydroxyapatite. The index ellipse of the crystallite system is indicated by the axes n_e and n_o. (b) The distribution of organic elements. A keratin-like protein material is concentrated around the margins of the prisms, but smaller amounts occur throughout

to an additional optical effect. A system of rod-shaped elements, such as crystallites, separated from each other by spaces containing a medium of different refractive index, constitutes a 'mixed body'[3]. A rodlet mixed body produces positive form birefringence relative to the direction of orientation of the rods. It follows, therefore, that enamel which is being formed or which is undergoing breakdown will show negative intrinsic birefringence due to its oriented inorganic component and positive form birefringence due to the intercrystallite spaces[4].

The strength of the positive form birefringence depends on the difference between the refractive indices of the crystallite material ($n = 1{\cdot}62$) and that of the medium occupying the spaces between the crystallites. The latter may be altered by the inclusion in these spaces of, for example, air ($n = 1{\cdot}0$), water ($n = 1{\cdot}33$), organic liquids such as alcohols ($n \sim 1{\cdot}4$) or quinoline ($n = 1{\cdot}62$)[2]. Quinoline has the same refractive index as the crystallite material. If it is used as the imbibing medium, form birefringence will not be produced and only the negative intrinsic birefringence of the mineral will be apparent. If the spaces are occupied by a medium of lower refractive index than this, then the result will be a summation of the positive form and the negative intrinsic birefringences. With media of refractive index 1·45, these opposing effects tend to compensate each other so that the system shows no activity between crossed polars. With media of lower refractive indices than this, the positive form birefringence predominates.

Using this method it has been possible to examine the nature of the spaces present in certain parts of the carious lesion (Fig. 2), and it has been found that the body of the lesion, which develops at a relatively late stage in the attack, behaves in exactly the way described[2]. However, one of the earliest stages of the carious lesion is the appearance of an opaque zone when examined in Canada balsam. This is the so-called dark zone. The development we wish to report concerns the detailed structure of the dark zone and the determination of the relative sizes of the spaces which are present, by showing that the extent of inclusion of organic liquids into these spaces depends on their molecular dimensions. It should, perhaps, be pointed out that whatever the size of spaces in the dark zone, the total volume of spaces is very small[5].

The dark zone is observed only in certain circumstances, for example when a tooth section is examined in media such as Canada balsam or quinoline (Fig. 2). It is also seen when a section is examined in air, but is not easily distinguished because it is concealed by the form birefringence of the neighbouring parts of the lesion. The dark colour of the zone as seen in transmitted light, as well as the strong positive birefringence seen between crossed polars, must be due to the fact that the intercrystallite spaces contain air. This suggests that the occurrence of the zone in sections mounted in Canada balsam or in quinoline is because the molecules of these liquids are too large to be included in the spaces.

This hypothesis has been subjected to experimental scrutiny by examining the optical behaviour of sections of carious lesions in enamel which were first dried and then allowed to imbibe, in turn, the liquids listed in Table 1. The results of a typical experiment are recorded there.

It will be noticed that the degree of observed birefringence becomes progressively more positive as the series of normal (straight-chain) alcohols is ascended from methyl alcohol to *n*-octyl alcohol. This effect cannot be due to differences in the refractive indices of the alcohols since the refractive index is approximately the same in each case. In fact the refractive indices do show a slight increase as the series is ascended. If the changes in refractive index had been influencing the total birefringence, then this would have produced a trend opposite to that observed. It therefore follows that this change in positive birefringence in the series, methyl

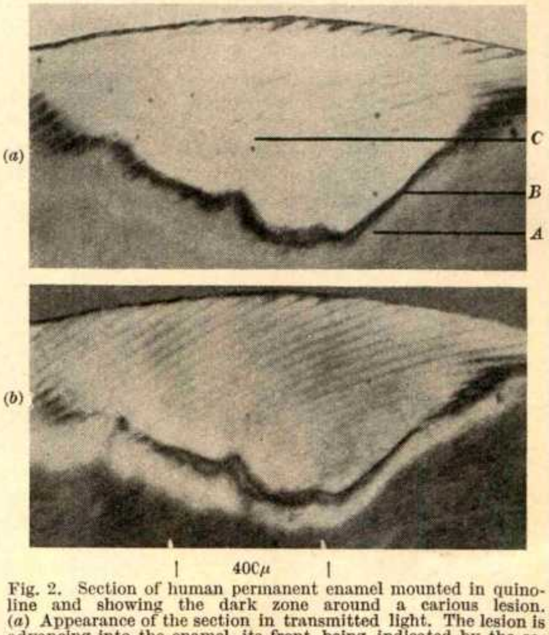

Fig. 2. Section of human permanent enamel mounted in quinoline and showing the dark zone around a carious lesion. (a) Appearance of the section in transmitted light. The lesion is advancing into the enamel, its front being indicated by the so-called translucent zone (*A*). Within the translucent zone is the deep brown, opaque 'dark zone' (*B*), and within this again is the body of the lesion (*C*). (b) Appearance of the same area of enamel between crossed polars. The dark zone shows up as a narrow band with positive birefringence and is delineated on either side from neighbouring areas by black lines of compensation. The rest of the enamel has negative birefringence

Table 1

Medium	Refractive index	Observed birefringence × 10^4
Methyl alcohol	1·33	− 11·97
Ethyl ,,	1·36	− 10·99
n-Propyl ,,	1·38	− 7·33
n-butyl ,,	1·40	− 5·85
n-Pentyl ,,	1·41	− 4·84
n-Hexyl ,,	1·42	+ 2·23
n-Heptyl ,,	1·42	+ 4·11
n-Octyl ,,	1·43	+ 5·85
Allyl ,,	1·41	− 9·71
*Iso*propyl ,,	1·37	+ 3·89
Tert.-butyl ,,	1·38	+ 11·39
Sec.-butyl ,,	1·39	+ 11·97
Tert.-amyl ,,	1·41	+ 12·59
Water	1·33	− 13·01
Quinoline	1·62	+ 13·21

→*n*-octyl alcohol, is due to changes in the extent of replacement of air ($n = 1{\cdot}0$) by alcohols ($n \sim 1{\cdot}4$) in the intercrystallite spaces. As the homologous series is ascended, the molecular size of the alcohols increases. It is suggested that, with any particular alcohol, spaces larger than a certain critical size can be occupied by the alcohol, whereas spaces smaller than this size cannot be occupied and will still contain air. In all the many specimens examined to date, a dark zone has never been observed in methyl alcohol. It may therefore be concluded that the smallest size of intercrystallite space so far detected is such as to allow the inclusion of molecules of methyl alcohol.

It has also been shown (Table 1) that branched-chain alcohols (for example, *iso*propyl or *tert.*-butyl alcohols) are included to different extents from the corresponding normal alcohols. This suggests that the ease of penetration depends not only on the linear dimensions but also on the cross-sectional areas of the intercrystallite spaces.

It should be emphasized that there is a variation in the sizes of the intercrystallite spaces so that with the higher alcohols only large spaces can be occupied, whereas with alcohols of smaller size, a greater number of spaces can be occupied. The extent of inclusion of the various liquids is an interesting demonstration of a molecular sieve phenomenon[6] in a biological system. Similar behaviour has also been observed in the non-carious tooth enamel of a variety of vertebrate animals. In each case the tissue showing the phenomenon appears to contain slightly less mineral than the fully formed normal enamel.

The significance of these results in relation to the understanding of the processes of carious breakdown will be discussed elsewhere[7].

This work was carried out while two of us (K. V. M. and D. F. G. P.) were in receipt of grants from the Medical Research Council, to which thanks are offered.

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Thread-like Reticulum in Latex from *Hevea brasiliensis* and its Relation to Latex Particles

IN a previous communication[1] I directed attention to the complexity of the 'lutoids' separated from fresh latex by high-speed centrifuging. I have now made further studies of fresh latex using phase microscopy. As reported in the literature[2-4], aggregates of lutoids occur in untreated fresh latex; I now find these aggregates not only contain lutoids, but also Frey Wyssling[5] particles and pear-shaped rubber particles, all of which occur in a gelatinous matrix. Associated with this gelatinous material, I have observed many very fine threads which often trail from the edges of the aggregates. These are quite distinct from the chains of coagulated rubber which have been reported in latex under certain conditions. Since the particles differ in density, the effect of high-speed centrifuging on the aggregates will be disruptive.

Latex was collected into chilled glass vessels and centrifuged under refrigerated conditions into zones by a technique essentially similar to that of Moir[6]. Moir remarks that the (middle) serum fraction often has a 'ropey' appearance to the naked eye. I now find this is due to many long thread-like processes beaded with particles including lutoids, Frey Wyssling particles, a few rubber particles and many particles too small to be identified. The Frey Wyssling particles commonly occur in grape-like clusters. In positions corresponding to Moir's zone 2 and zone 11 are found patches of translucent jelly. The zone 11 jelly at the bottom of the tube contains many particles, especially lutoids, and sometimes portions of plant tissue. The zone 2 jelly in the upper half of the tube contains threads to which many rubber particles are attached. The general appearance is that of fruit growing from a branch. Pear-shaped rubber particles are attached by their stalk-like narrow ends. Apparently the aggregates seen in untreated latex have been torn apart, rubber particles moving upwards and lutoids downwards, with attached threads and jelly parted between them. A portion remains incompletely separated, intermediate in density, and is found in the serum. Frey Wyssling particles are found in all three regions.

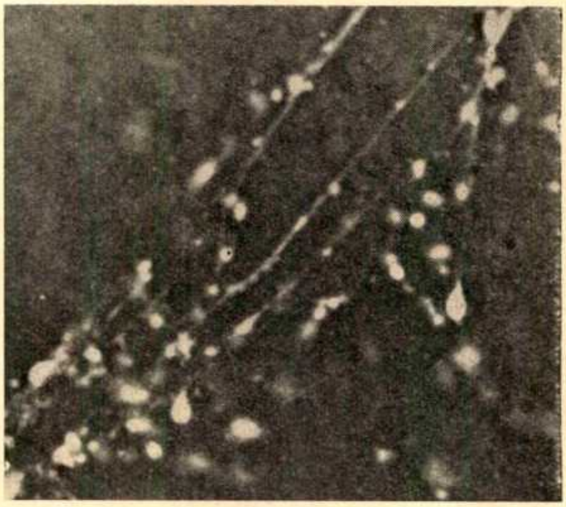

Fig. 1. Rubber particles attached to threads in upper (zone 2) fraction. Lutoids and Frey Wyssling particles have been stripped from the threads by the centrifuging. Reichert negative phase, flash illumination. (× *c.* 2,530)

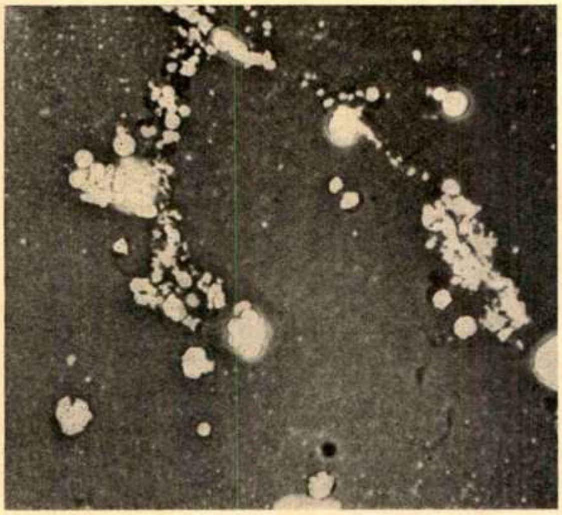

Fig. 2. Partially disrupted aggregates in the middle (zone 5/6) fraction. Rubber, lutoids, clusters of Frey Wyssling particles are still attached. Reichert negative phase, flash illumination. (× c. 1,330)

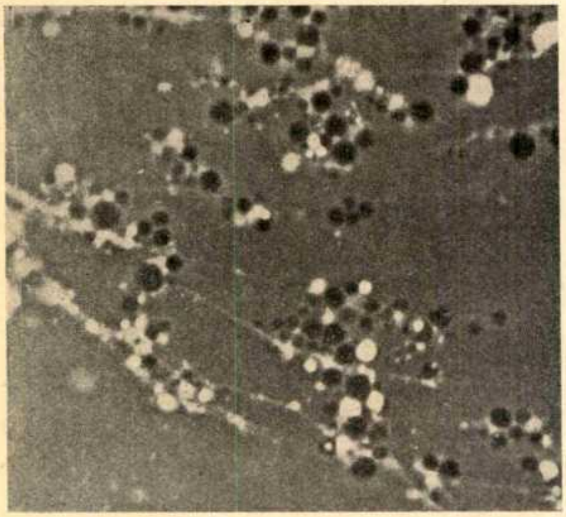

Fig. 3. Lutoids attached to threads in lower fraction from re-centrifuged aggregates. Rubber and most Frey Wyssling particles have been stripped from the threads by the centrifuging. Reichert negative phase, flash illumination. (× c. 1,330)

As supposed by Moir[6], zone 8 is predominantly lutoids. Careful examination shows that some of these have short lengths of thread attached, suggesting that they have been torn from the main thread system. By re-centrifuging serum containing partially separated aggregates, I have been able to concentrate such lutoids. Using time-lapse photography I have recorded interactions between thread and lutoid. Over a period of 2 hr. the thread thickens and proves to be tubular, for the spherical lutoid suddenly discharges its contents into the swollen thread, the whole forming an elongated body enclosing captive particles in motion. A similar experiment with a short length of thread studded with very small particles recorded a swelling which formed locally. The small particles were then seen to be freely in motion within the swelling, so that they must have been contained originally inside the tubular thread and not attached to its exterior. The swollen thread had the appearance of a lutoid complete with captive particles.

From these experiments it seems that the aggregates include all three types of particle commonly seen in latex, linked by a reticulum of fine threads now shown to be tubular and sometimes to enclose very small particles in their interior. The whole constitutes a rather jelly-like matrix and is probably a fragment of a more extensive cytoplasmic structure in the latex vessels, carried away by the outflow of latex. Such a reticulum is, of course, a most likely site for particle synthesis. Normal tapping methods must tear many particles free from their attachments even if they are not liberated by other means. Frey Wyssling's observation[5], that the particles called after him often occur in clusters, is confirmed. It now seems that they are formed in this fashion on the reticulum. Similarly the characteristic pear shape of many rubber particles[7,8] follows from their mode of formation, whether or not the aggregation mechanism proposed by Lucas[9] operates afterwards.

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CHEMISTRY

'Ligand Exchange': a Novel Separation Technique

A novel technique for analytical and preparative separations of compounds which can form complexes or adducts with metal ions has been worked out in this laboratory. The new technique can appropriately be called 'ligand exchange'. The method combines two fields of chemistry, namely, ion exchange and co-ordination chemistry, in order to accomplish a task that neither could do alone.

An ion exchanger containing a complexing metal ion (Cu^{2+}, Cu^{+}, Ni^{2+}, Ag^{+}, Co^{3+}, etc.) is used as a solid sorbent. The potential ligands are sorbed from solutions or gases and form complexes with the metal in the resin or displace other ligands which have previously complexed the metal. The latter remains in the ion exchanger. In contrast to conventional ion exchange where counter ions are exchanged, ligand exchange is exchange of ligands for others or for solvent molecules in the ionic solvation shells. Ligands which can be isolated or separated include ammonia, organic amines, polyhydric alcohols, olefins, acetylene derivatives, etc., and anions of organic acids and amino-acids. Separations are preferably carried out in a chromatographic column as in ion exchange chromatography or gas-liquid chromatography. Standard chromatographic techniques—elution development, gradient elution, displacement development, frontal analysis, etc.—can be adapted to ligand exchange.

Ligand exchange has several advantages over other, more conventional chromatographic techniques. First, complex formation is a very strong interaction if the proper metal ion is chosen. This provides a very strong 'driving force' for ligand sorption. Thus, ligand exchangers sorb ligands selectively, utilizing almost their full capacity even when the ligand concentration in the external solution or gas phase is extremely low. In this way, ligands can be very efficiently separated from non-ligands. For example, from 10 litres of a 10-p.p.m. solution of most aliphatic amines, essentially all is sorbed by 1 gm. or less of a cation exchanger in Cu^{2+} or Ni^{2+} form. Secondly, complex formation is a highly specific interaction, much more so than ordinary physical adsorption or ion exchange. Complexes of a metal ion with various, even rather similar, ligands differ greatly in their strength. Hence the ligand exchanger can distinguish rather sharply between such ligands, and outstandingly sharp resolutions in separations of ligands from one another can be obtained even in cases in which conventional sorbents fail. A striking illustration of the specificity of complex formation is the separation of the rare earths by ion exchange chromatography; here, differences in complex-strengths with an eluting or developing agent are used for separating metals. Ligand exchange is a reversal of this technique; differences in complex-strengths with a metal are used for separating complexing agents. For example, with ammonia and an organic diamine, separation factors well above 100 were easily attained. Thirdly, ligand exchange is very flexible since the metal ion giving the best separation can be chosen from a great number of elements.

A unique and particularly useful feature of ligand exchange is the following. In separations of ligands of different co-ordinative valences the selectivity can be adjusted and even reversed at will, simply by varying the total ligand concentration in the external solution or gas phase. Consider, for example, the 'ligand exchange' of a monodentate ligand A (occupying one co-ordinative valence of the metal) for a bidentate ligand B (occupying two co-ordinative valences) with, say, Ni^{2+} as the complexing metal ion:

$$\mathrm{Ni}A_6 + 3B \rightleftharpoons \mathrm{Ni}B_3 + 6A \qquad (1)$$

The mass action law for this exchange can be written as:

$$\frac{[\mathrm{Ni}B_3][A]^6}{[\mathrm{Ni}A_6][B]^3} = K_a = \text{const.} \qquad (2)$$

or

$$\frac{[\mathrm{Ni}B_3][A]^3}{[\mathrm{Ni}A_6]} = K\left(\frac{[B]}{[A]}\right)^3 \qquad (3)$$

For a given constant concentration ratio of B to A, the right-hand side of equation (3) remains constant. Thus, high total ligand concentration (high value of $[A]$ on the left-hand side) favours the complex $\mathrm{Ni}A_6$, and low total ligand concentration (low value of $[A]$) favours the complex $\mathrm{Ni}B_3$. By selecting a suitable metal ion one can adjust conditions in such a way that the monodentate ligand is preferentially sorbed from concentrated solutions and the bidentate one from dilute solutions. For example, a bidentate diamine is economically separated from (monodentate) ammonia by selective sorption of the diamine from a dilute mixture by a cation exchanger in metal–ammonia–complex form. The diamine is then recovered from the column by displacement with concentrated ammonia, which yields the diamine in high concentration. No regeneration is required since, after displacement, the resin is again in metal–ammonia–complex form.

Displacement of the complexing metal ion from the resin by ion exchange with other cations in the external solution interferes with ligand exchange. Such ion exchange can be suppressed by choosing a resin with ionogenic groups which hold the metal firmly by partial complexing. In non-ionic ligand exchange, carboxylic acid groups usually offer sufficient protection. If requirements are more stringent, as is the case in anionic ligand exchange, chelating resins such as 'Dowex A-1' (iminodiacetic acid groups) can be used. A possible alternative is the use of an anion exchanger with amino groups. Of course, such protection against ion exchange is achieved at a sacrifice in ligand exchange capacity since the ionogenic groups block co-ordinative valences of the metal ion. For example, with Ni^{2+}, two co-ordinative valences out of six are blocked in resins with carboxylic acid groups, and three in resins with iminodiacetic acid groups.

Ligand sorption by cation exchangers containing complexing metal ions was observed as early as 1954 by Walton[1]. Separations by virtue of differences in complexing strength have occasionally been achieved by other authors[2]. However, it appears that so far the general applicability of the principle, the inherent advantages, and, in particular, the effect of the co-ordinative valences of the ligands have not been recognized.

Separation factors and column performance in ligand exchange operations can be predicted with reasonable accuracy solely on the basis of the tabulated values of the complex-formation constants. Publication of a theoretical treatment and experimental results is anticipated elsewhere.

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Method for studying Thermal Stability of Bulk Thermosetting Polymers

THE use of thermosetting polymers in high-temperature applications is increasing rapidly; but data on the thermal stability and degradation characteristics of these materials are very limited. A few investigations employing differential thermal analysis and thermogravimetric analysis have been reported, but the number of polymers investigated is small. Both methods, while very useful in elucidating the kinetics of the degradation process, require a specially prepared sample and are not suited to yield information on bulk polymers. A new experimental technique, termed electro-thermal analysis, for determining thermal stability of bulk thermosetting polymers, will be described briefly.

Previous work with electrical volume-resistivity measurements[1,2] has shown their usefulness in studying reactions and transitions of cross-linked polymers. It has also been shown[2] that a completely polymerized

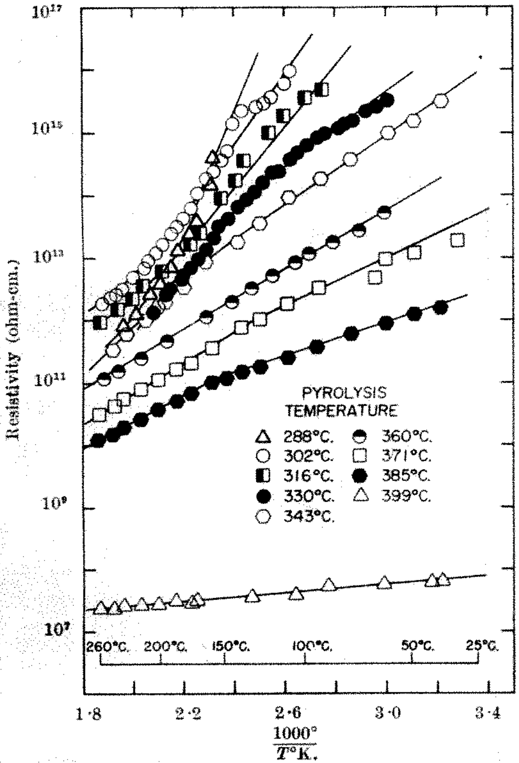

Fig. 1. Temperature-dependence of the electrical resistivity of a thermosetting polymer after pyrolysis for 16 hr. at various temperatures

polymer will exhibit not only the maximum magnitude of resistivity but also the maximum temperature-dependence of resistivity. A cross-linked organic polymer will on pyrolysis exhibit a progressive decrease in these parameters due to degradation, the magnitude of the decrease being approximately proportional to the extent of degradation. At 25° C. the magnitude of the resistivity, $\rho(25°)$, will approach as a limiting value the resistivity of carbon, that is, about 10^5–10^7 ohm-cm., while the temperature-dependence will approach the flat or slightly negative dependence characteristic of carbon. Pyrolysis progressively converts a cross-linked organic polymer into a dense, rigid, extensively conjugated, cross-linked carbonaceous residue[3,4]. This progressive pyrolytic conversion can be measured by resistivity techniques and forms the basis for this investigation. With the exception of a limited study of the structure of several pyrolysates by Winslow and co-workers[3], the use of resistivity measurements in this manner has not been previously reported.

Degradation is determined by repeatedly measuring the temperature-dependence of the resistivity over the range 25–300° C. after each pyrolysis cycle. The arbitrarily chosen pyrolysis cycle is 16 hr. and the temperature is increased about 15 deg. C. for each cycle. Dependence on temperature can be conveniently determined by measuring the resistivity as the pyrolysate is slowly cooled. Onset of thermal degradation is shown by a decrease in the previously established maximum magnitude of resistivity and its dependence on temperature. Pyrolysis is then conducted at progressively higher temperatures until the residue exhibits the electrical properties of carbon. Pyrolysis at still higher temperatures results in graphitization of the carbon and in decreased resistivity[4].

Resistivity measurements are made by a continuous-current monitoring device already described[1]. Parallel-plate electrodes are employed which are completely embedded in the polymer, and pyrolysis is thus conducted in the absence of air. The initial temperature of pyrolysis is a function of the polymer under consideration but will usually be greater than 200° C.

Results obtained by this technique on a fluorinated hydrocarbon thermosetting polymer are shown in Fig. 1. This is a semilogarithmic (Arrhenius) plot of resistivity versus temperature of a polymer after pyrolysis at progressively higher temperatures. This polymer exhibits the maximum values of $\rho(25°)$ and temperature-dependence after heating at 288° C. After pyrolysis at 302° C., a progressive decrease occurs in both parameters. The curve for temperature-dependence becomes almost flat after pyrolysis at 399° C. The large change in $\rho(25°)$ and temperature-dependence between the pyrolyses conducted at 385° and 399° C. suggests that the last significant amount of organic polymer has been burned off, leaving a residue which is almost entirely carbon.

Starting with nickel- and aluminium-doped ion exchange resins, Pohl and co-workers[5] have prepared semiconducting 'pyro-polymers' by this pyrolytic technique.

This technique is used in conjunction with differential thermal analysis and thermogravimetric analysis in investigations of the high-temperature characteristics of various polymers. It is suggested that it be standardized as a test method for determinations of thermal stability on bulk thermosetting polymers. Additional data on epoxide, epoxylated-novolac, polyurethane and silicone polymers together with a discussion of the degradation process in bulk thermosetting polymers will be published later.

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Complexes of Zerovalent Vanadium, Chromium, Molybdenum and Tungsten with a Ditertiary Phosphine

We have previously reported that compounds of type [Mo(diphosphine)$_3$] are obtained from the reaction between dibenzenemolybdenum and the appropriate ditertiary phosphine[1], but we were unable to obtain analogous compounds of chromium by this method. We have therefore developed a direct method of preparation which, in addition to the molybdenum compound, has supplied the $Me_2PCH_2CH_2PMe_2$ complexes of vanadium(0), chromium(0) and tungsten(0) as listed in Table 1. These compounds were obtained by reducing a mixture of the metal chloride and three equivalents of the ditertiary phosphine in dry tetrahydrofuran. Lithium aluminium hydride (*A*) and sodium naphthalide (*B*) were used to effect reduction.

Table 1

Compound	Colour	m.p. (decomp.)	Reagent	Metal chloride
$[Cr\{C_2H_4(PMe_2)_2\}_3]$	bright yellow	309°	*A* or *B*	$CrCl_3$.3tetrahydrofuran
$[Mo\{C_2H_4(PMe_2)_2\}_3]$	yellow	397	*A* or *B*	$MoCl_5$*
$[W\{C_2H_4(PMe_2)_2\}_3]$	pale yellow	390	*A*	WCl_6*
$[V\{C_2H_4(PMe_2)_2\}_3]$	deep brown	254	*B*	VCl_3

* Reduced in tetrahydrofuran to 3-valent state before addition of ditertiary phosphine.

Reduction appears to be stepwise. For example, the VCl_3-diphosphine solution is deep green, and as reduction proceeds the colour changes through lavender blue to pale brown and is finally deep brown.

The compounds oxidize very quickly in air, and are decomposed by hydroxylic solvents, but have surprising thermal stability. They were purified by recrystallization from hot benzene, in which they are only sparingly soluble. The infra-red spectra of the compounds in 'Nujol' mulls are almost identical. $[V\{C_2H_4(PMe_2)_2\}_3]$ is paramagnetic ($\mu = 2{\cdot}10 \pm 0{\cdot}25$ B.M.), and its electron paramagnetic resonance spectrum is being investigated by Dr. J. F. Gibson of these Laboratories.

A full account of these and of similar compounds of other metals will be published later.

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Stereochemistry of the Succinic Dehydrogenase System

IN continuation of work[1-3] on the stereochemistry of Krebs-cycle reactions, the stereochemistry of the succinic dehydrogenase reaction has been investigated with substrates that constrain the enzyme system to demonstrate its stereospecificity. These substrates are L-chlorosuccinic acid, newly found by us, and the L-enantiomorph of methylsuccinic acid; the racemic modification of the latter having been previously demonstrated[4,5] to decolorize methylene blue in the crude heart preparations.

Fig. 1 presents typical kinetic curves obtained by the Warburg technique for the uptake of oxygen by L-chlorosuccinic acid and L-methylsuccinic acid in the presence of Slater's succinic dehydrogenase preparation[6]. It is to be noted that the uptake of oxygen by L-chlorosuccinic acid is of the same order as that for succinic acid while that for methylsuccinic acid is decidedly less. The products of the oxidation were isolated and identified by chromatography[2] from the residue obtained by continuous ether extraction of acidified and deproteinized reaction mixtures. These products were, respectively, chlorofumaric acid and mesaconic acid (methylfumaric acid), thus establishing the dehydrogenation nature of the reaction and the fact that substituted fumaric acids results from the reaction. Of further interest is the fact that D-chlorosuccinic acid has but slight acitivity as a substrate and that D-methylsuccinic acid is not a substrate. The latter compound has been investigated for inhibitory properties by a spectrophotometric technique[7] and is a competitive inhibitor of succinate oxidation, K_I having the value $1{\cdot}2 \times 10^{-2}$ *M*, at *p*H 7·3 and 35·5°.

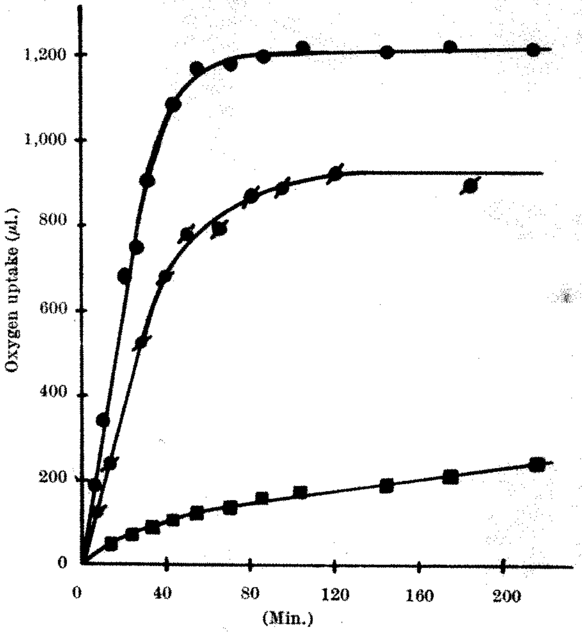
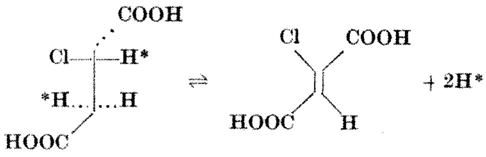
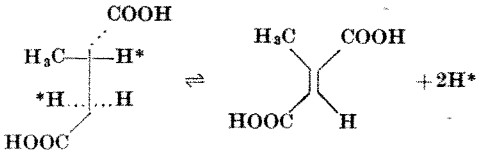


Fig. 1. Warburg oxidation of α-substituted succinic acids by succinic dehydrogenase at *p*H 7·3, 35·5°. Each reaction mixture contained 2·0 ml. succinic dehydrogenase suspension[6] and was 0·056 *M* in phosphate buffer, $6{\cdot}6 \times 10^{-4}$ *M* in methylene blue and 4×10^{-3} *M* in cyanide; total volume, 4·5 ml. ●, 0·018 *M* succinate; ⧫, 0·027 *M* L-chlorosuccinate; ■, 0·054 *M* L-methylsuccinate, 85 per cent optical purity

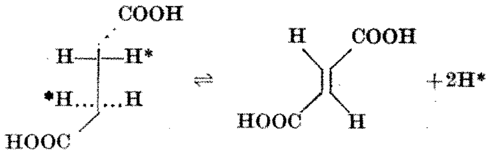
It is clear from these results that the succinic dehydrogenase system is, as expected, asymmetric in its action albeit this asymmetry cannot be detected with succinic acid by ordinary techniques[8]. The above results in conjunction with the recently, seemingly conclusive, demonstrated[9] trans-stereoselectivity of the succinic dehydrogenase system permits steric depiction of the reactions, as follows:



(1)

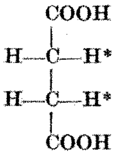


(2)



(3)

where the starred hydrogen atoms are those removed by the enzyme from L-chlorosuccinic acid (1) and L-methylsuccinic acid (2), while for succinic acid (3) the starred hydrogens are those that are removed from the enzyme-substrate complex, that is, the succinic acid molecule depicted may be rotated 180° around an axis perpendicularly bisecting the bond between the two α-carbon atoms and then placed in the enzyme to yield an enzyme-substrate complex that would lose the two unstarred hydrogens. The two hydrogens that are stereospecifically and stereoselectively removed from the enzyme-substrate complex may be called D-hydrogens, according to the projection formula:



and it is again to be noted that enzymes confer asymmetry on non-asymmetric molecules[10].

It is of interest to note that the succinic dehydrogenase system operates on L-substrates in a *trans* fashion as do *cis*-aconitase[2] and fumarase[3], and that the stereochemistry of the Krebs cycle may now be considered complete since the stereochemical pathway from fumaric acid to *iso*citric acid has been previously traced[8].

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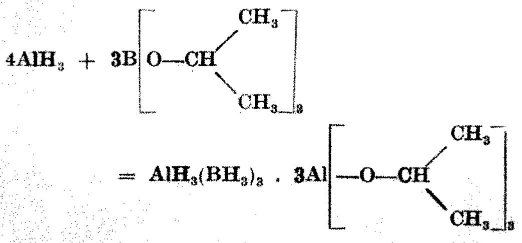
A New Class of the Complex Hydrides

While studying the reaction of aluminium hydride with esters of boric acid, the formation of a new type of the complex hydrides was observed. Thus, aluminium hydride reacts with *iso*propyl borate in ethyl ether, giving rise to a typical representative of the new type of hydride compounds:



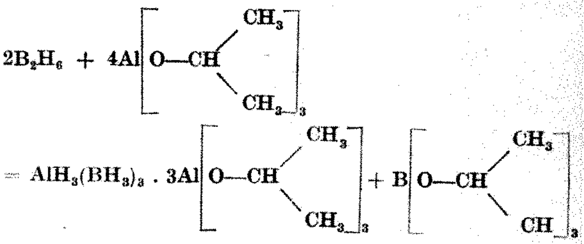
(I)

The product is a colourless liquid, distillable *in vacuo* (boiling point at about 120° C. at 0·5 mm. pressure, without decomposition). The yield is about 85 per cent. Analysis: $B_3Al_4H_{75}O_9C_{27}$ (molecular weight, calculated 684·25). B, 4·58 per cent; Al, 15·7 per cent; H, 11·05 per cent; H_{active} (assay by decomposition with diluted hydrochloric acid), 1·79 per cent. C, 45·95 per cent. Calculated values: B, 4·73 per cent; Al, 15·7 per cent; H, 11·01 per cent; H_{active}, 1·76 per cent; C, 47·3 per cent. Complex formation between complex borohydrides and alcoholates is not recorded in the literature, although the possibility of the formation of mixed crystals of lithium borohydride with lithium methoxide has already been considered[1].

The nature of the bonding in these new complexes is not known; their structure assigned above is confirmed by an alternate synthesis of (I) by the reaction of aluminium borohydride[2] with aluminium *iso*propoxide in tetrahydrofuran. Borane aluminium alcoholate is proposed as a generic name for these compounds. Thus (I) is borane aluminium *iso*propoxide. These compounds display a varying degree of sensitivity to air; they are handled preferably under pure nitrogen. The reaction is analogous to that of aliphatic or alicyclic borate esters, but with borate esters of phenols a different reaction occurs:

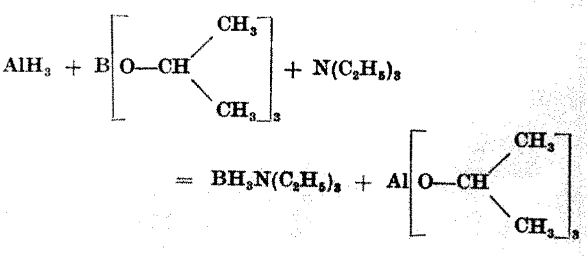
$$2AlH_3 + 2B(-O-C_6H_5)_3 = B_2H_6 + 2Al(-O-C_6H_5)_3$$

The diborane formed evolves from the ethereal reaction mixture after warming it up to 35° C. in a yield of about 70–80 per cent. Compounds of type (I) can be obtained with a 'reversed' method also. For example, diborane and aluminium *iso*propoxide react in tetrahydrofuran, giving rise to (I) and *iso*propyl borate:



This reaction is similar to that of diborane with the alcoholates of the alkaline metals[3].

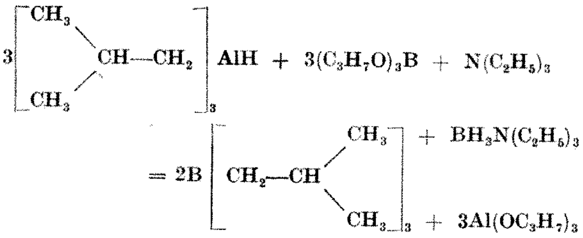
Sodium methoxide and lithium ethoxide give, with diborane, lithium borohydride and sodium borohydride, respectively. H. I. Schlesinger and co-workers treated aluminium alcoholates with diborane gas, but, using solid alcoholates, they obtained in a partial reaction an uncharacterized mixture of different products. The presence of amines completely changes the picture. Thus, aluminium hydride gives, with borate esters, in the presence of triethylamine triethylamine-borane (yield about 75 per cent) and aluminium alkoxide:



Borane aluminium *iso*propoxide is a strong reducing agent. It reduces many types of organic and inorganic compounds, for example, the carbonyl compounds to the corresponding alcohols, the esters to the primary alcohols, the nitriles to the amines. Its reducing strength probably equals the strength of aluminium borohydride. What seems important is that it is soluble and can be used in a broad range of solvents, for example, hexane, ligroin, mineral oil, ethyl ether, chloroform. No other hydride-type reducing agent is soluble in such a broad range of solvents.

The covalent, non-salt-like character and the strong reducing ability of these compounds agree with the correlation recognized earlier between these properties of the complex borohydrides[4].

As I found, the reaction of aluminium alkyl hydrides with borate esters gives products indicating that these compounds behave as mixtures of aluminium hydride and aluminium alkyls. For example, di-*iso*butyl aluminium hydride in the presence of triethylamine reacts with *iso*propylborate, giving rise to tri-*iso*-butylboron and triethylamine borane[5]:



$$3[(CH_3)_2CH{-}CH_2]_3AlH + 3(C_3H_7O)_3B + N(C_2H_5)_3 = 2B[CH_2{-}CH(CH_3)_2]_3 + BH_3N(C_2H_5)_3 + 3Al(OC_3H_7)_3$$

The complex aluminohydrides, for example $LiAlH_4$ and $NaAlH_4$, are transformable also, by treating them with borate or metaborate esters, to the corresponding borohydrides :

$$LiAlH_4 + B(OR)_3 = LiBH_4 + Al(OR)_3$$

The yields are 70–90 per cent; patients have been applied for.

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BIOCHEMISTRY

Evaluation of the Activity of Corticosteroid 11β-Hydroxylase

2-METHYL-1,2-*bis* (3-pyridyl)-1-propanone (*SU* 4885) has been introduced as an agent for assessing pituitary–adrenal integrity and pituitary reserve[1]. The action of *SU* 4885 is to inhibit 11β-hydroxylation on the corticosteroid nucleus[2,3]. Administration of *SU* 4885 in the presence of a normal pituitary–adrenal relationship results in an increased excretion of 17,21-dihydroxy-20 ketosteroids as measured by the Porter–Silber[1] and ketogenic steroid reactions[4]. This increase is a resultant of the combined effect of decreasing 11β-hydroxylation, presumably increasing adrenocorticotrophic hormone production and increasing the production of 11-deoxycorticosteroids.

Since the inhibition of 11β-hydroxylation is not complete, situations may arise where there may not be a marked change in the total corticosteroid excretion even though there may be a positive adrenal response to *SU* 4885. In such cases the inhibition of the 11β-hydroxylase may still be present, and if one were to measure this inhibition more directly, it might be possible to determine whether there is a response to *SU* 4885 when the change in total 17,21-dihydroxy-20 ketosteroids is not marked. Where total corticosteroid excretion fails to rise, this may be due to failure of the *SU* 4885 to penetrate the adrenocortical cells, or to produce inhibition once it is there. An examination of the values of the 11 oxygenated steroids may present information on this point.

Evaluation of the action of *SU* 4885 should take into consideration the 11-oxy steroids as well as the 11-deoxy steroids. It is possible to separate 11-oxy from 11-deoxy 17-ketosteroids by employing column chromatography on alumina[5]. By means of the ketogenic reaction[6] the 17-OH-corticosteroids may be converted to 17-ketosteroids. These 17-ketosteroids may then be separated into the 11-oxy and 11-deoxy groups and each determined quantitatively by the Zimmermann reaction. The preformed 17-ketosteroids present in the urine are first reduced to alcohols by means of sodium borohydride in order to eliminate them from measurement. We have been utilizing the chromatographic method for the separation of 11-oxy ketosteroids from 11-deoxy ketosteroids and have combined this with the ketogenic reaction to study the effect of *SU* 4885 on human beings.

By expressing the results as the ratio of 11-deoxy : 11-oxy 17-ketogenic steroids, one has a more sensitive measurement of the action of *SU* 4885 than a measurement of total 17-OH-corticosteroids. Table 1 illustrates this by giving the values obtained for various individuals for the total ketogenic steroids and 11-deoxy : 11-oxy 17-ketogenic steroid ratios. It will be seen that even in cases where there were no marked increases in total ketogenic steroids, there were good-sized increases in the ratios. In case 5, there was a rather marked but unexplainable fall in total ketogenic steroids after the administration of *SU* 4885. There was, however, an increase in the 11-deoxy : 11-oxy 17-ketogenic steroid ratio. This technique of measuring the change in ratio may be a

Table 1. EFFECT OF *SU* 4885 ON TOTAL 17-KETOGENIC STEROIDS (KGS) AND 11-DEOXY : 11-OXY 17-KETOGENIC STEROID (KGS) EXCRETION IN URINE

Patient	Control before *SU* 4885		After *SU* 4885‡	
	KGS (mgm./24 hr.)	11-Deoxy-KGS / 11-Oxy-KGS	KGS (mgm./24 hr.)	11-Deoxy-KGS / 11-Oxy-KGS
1	7·1	1·6	15·8, 18·1	2·3, 4·4
2	10·6	1·4	27, 40	2·8, 3·9
3	9·6	1·5	11·9	2·7
4	15·5, 11·8 12·1	1·5, 1·7 2·0	14·2, 16·8	2·5, 2·4
5	29, 21 15·7	1·9, 2·4 2·3	8·5, 9·2	4·1, 4·2
6*	25	2·5	35, 45, 49	3·5, 2·8, 3·3
7†	4·3	1·0	2·9, 1·9, 2·1	1·5, 1·0, 1·9

Patients 1–5 were adult males who were hospitalized for non-endocrine disorders.

* This patient was a case of hyperadrenalism, possibly tumour.

† This patient was a case of hypopituitarism.

‡ *SU* 4885 was administered orally 6 × 0·5 gm. daily.

relatively simple method for evaluating the action of *SU* 4885 where interpretation of results from determination of total corticosteroids may be ambiguous.

Recently, Butt *et al.*[7] have reported on a similar fractionation technique to investigate the effect of *SU* 4885. They utilized silica-gel chromatography to effect separation of the ketosteroids. Our results support the claim of Butt *et al.*[7] that the 'fractionation' method has greater sensitivity than the total 17-ketogenic steroid method.

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The Metabolic State of Thiosulphate

OUR earlier experimental work using labelled thiosulphate has shown that the two atoms of sulphur in this compound have different fates in the course of metabolism in the animal organism[1]. In rats, the inner sulphur atom is eliminated very quickly from the organism in the form of sulphate, while the outer atom is transformed into the sulphate much more slowly, going probably through a number of intermediate stages. The results of our investigations have shown that the thiosulphate may be the metabolic precursor of the sulphate, and that, however small its concentration in rat urine may be, the endogenous pool of thiosulphate is large (ref. 1*b*).

In the work reported here, rats were injected subcutaneously with 0·8 mgm. of D,L-cystine labelled with sulphur-35 dissolved in 0·5 per cent sodium carbonate, and at the same time with 200 mgm. non-radioactive sodium thiosulphate ($Na_2S_2O_3.5H_2O$). The dose of thiosulphate was repeated after 24 hr. The urine was collected quantitatively at set intervals of time. As a control, we used rats to which radioactive cystine had been given without the simultaneous administration of thiosulphate. The thiosulphate excreted in the collected urine was separated and estimated quantitatively by the method of Gast *et al.*[2], the residual sulphate was removed by adding barium chloride, and the remaining sulphur compounds which were neither thiosulphate nor inorganic sulphate were treated as residual sulphur. The total activity of a given sample of urine, the activity of the thiosulphate, and that of residual sulphur were determined. The activity of the inorganic sulphate was calculated indirectly from the difference.

Table 1. ACTIVITIES OF EXCRETED THIOSULPHATE, SULPHATE AND RESIDUAL SULPHUR

Results are expressed as a percentage of total activity of urine, after injection of 0·8 mgm. of cystine-^{35}S (4 animals)

Time (hr.)	Activity of thiosulphate		Activity of sulphate		Activity of residual sulphur	
	Range	Average	Range	Average	Range	Average
0–6	1·79–2·28	2·1	46·5–78·5	71·8	19·3–31·4	26·0
6–24	1·88–3·22	2·47	53·7–77	67·4	20·7–44·5	30·1
24–48	2·45–3·00	2·64	51·2–68·8	62·6	28·1–45·5	34·2
48–72	1·69–2·71	2·36	53–72	60·8	26·4–43·5	36·5

Table 2. ACTIVITIES OF EXCRETED THIOSULPHATE, SULPHATE AND RESIDUAL SULPHUR

Results are expressed as a percentage of total activity of urine, after injection of 0·8 mgm. cystine-^{35}S and 200 mgm. $Na_2S_2O_3.5H_2O$. The dose of $Na_2S_2O_3.5H_2O$ was repeated after 24 hr. (6 animals)

Time (hr.)	Activity of thiosulphate		Activity of sulphate		Activity of residual sulphur	
	Range	Average	Range	Average	Range	Average
0–6	38·5–53·2	43·6	31·7–51·8	44	12·0–15·0	13·5
6–24	1·9–19·5	7·6	56·7–77·0	65·5	21·2–30·0	25·4
0–24	29–41	33	47–52	49	12–19	18
24–48	10·2–30·9	18·95	30·0–60·4	51	19·7–39·2	28
48–72	2·5–7·3	5·3	42·5–57·7	52	37·0–52·5	42·2

Table 1 shows the proportion of radioactivity of the different fractions of sulphur in urine after giving rats cystine alone. Table 2 similarly illustrates the results of experiments when both radioactive cystine and non-radioactive thiosulphate were injected.

As may be seen from Tables 1 and 2 only an insignificant percentage of activity is excreted in the form of thiosulphate after the injection of labelled cystine without the addition of thiosulphate. On the other hand, if at the same time a massive dose of unlabelled thiosulphate is given, 33 per cent of the sulphur produced by the metabolism of cystine is excreted in the urine in the form of thiosulphate in the course of 24 hr. Even after the next 48 hr., considerable quantities of radioactive thiosulphate are excreted when additional thiosulphate has been injected.

Our explanation of these facts is that external introduction of a large dose of thiosulphate inhibits the oxidation of the endogenic thiosulphate. We assume that thiosulphate given from an external source mixes with the pool of endogenous thiosulphate, and in consequence both the excretion and the oxidation of the endogenous thiosulphate take place at the same rate as that of the thiosulphate administered from an external source. A scheme of the relation between the exogenous and endogenous thiosulphate is given in Fig. 1, where $*A_0$ is the sulphur of the endogenous thiosulphate; $*A$ is the sulphur of the endogenous thiosulphate excreted in the urine in the form of thiosulphate; $*B$ is the sulphur in the

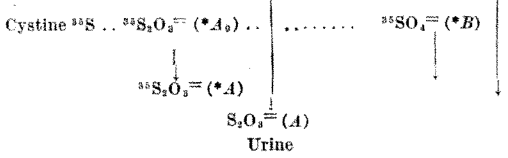

Fig. 1

sulphates originating from the radioactive cystine excreted in urine; A_0 is the sulphur of the injected thiosulphate; A is the sulphur of the injected thiosulphate excreted in the urine in the form of thiosulphate; B is the sulphur in the sulphates originating from the injected thiosulphate excreted in urine.

Quantitatively, if the endogenous and exogenous thiosulphate behave in an identical manner, the following relation would be valid: $^*A_0/^*A = A_0/A$.

We had previously estimated[1] that under normal conditions the rat excretes 0·05–0·1 mgm. of sulphur in the form of the thiosulphate and about 3 mgm. of sulphate sulphur during 24 hr.; that is, the ratio of these two kinds of sulphur may be expressed numerically as 0·01–0·03. The same relation exists between activity of thiosulphate and sulphate after injection of labelled cystine alone (Table 1). After injecting rats with labelled thiosulphate, we showed that the relation of excreted thiosulphate sulphur to the sulphate sulphur (A/B) is given by the average values 0·61, 0·19 and 0·53 for periods 0–6, 6–24 and 0–24 hr. respectively.

If we assume that thiosulphate is the normal precursor of the sulphate and that the small quantity in the urine is only the result of its rapid oxidation in the animal organism, after the injection of thiosulphate the ratio of the sulphur of the endogenous thiosulphate to the sulphur of the endogenous sulphate should be approximately the same as that of the introduced thiosulphate sulphur to the sulphate originating from this: $^*A/^*B = A/B$. Taking the figures from Table 2, we obtain the following values of $^*A/^*B$: 0·99, 0·11, 0·67 for periods 0–6, 6–24 and 0–24 hr. respectively.

The figures obtained for $^*A/^*B$ as compared with values of A/B lie in the range of experimental deviation and these results indicate with great probability that the endogenous thiosulphate behaves like the exogenous, and hence it is the fundamental precursor of the sulphate originating in the organism of the rat.

A full description of the experiments and calculations will be given elsewhere.

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Polysaccharides of *Mycobacterium phlei*

INVESTIGATIONS of the polysaccharides of *Mycobacterium phlei* have been few and mainly concerned with those secreted into the culture medium. Thus Masucci and McAlpine[1] found that a polysaccharide which they isolated from culture filtrates contained D-mannose and D-arabinose. Tennent and Watson[2] isolated a similar product and showed that the molecular weight was 7,300. In the present investigation the intracellular polysaccharides of *M. phlei* were studied.

The organisms were grown on a culture medium (10 litres) consisting of 'Oxoid' bacteriological peptone, 1 per cent; 'Lab. Lemco', 1 per cent; sodium chloride, 0·5 per cent, and glycerol, 5 per cent at 37° for 16 days. The cells were separated from the medium, washed with 0·14 *M* sodium chloride, suspended in phosphate buffer, *p*H 8, and disintegrated in batches of 250 ml. by shaking at 0° for 5 hr. with ballotini glass balls (1 mm. diameter) in a vibratory ball mill. The suspension of broken cells was diluted to 1 litre with phosphate buffer, *p*H 8, and then adjusted to *p*H 4 with dilute acetic acid. The suspension was centrifuged at 2,600 r.p.m. at 0° and the precipitate (cell debris, nucleoproteins, etc.) discarded. The supernatant liquids from the various batches were combined, concentrated to 1 litre by freeze-drying and then mixed with 5 volumes of ethanol. The precipitate so produced was centrifuged off, dissolved in distilled water, dialysed and freeze-dried to give a crude polysaccharide fraction (12 gm.), $[\alpha]_D^{20} + 164°$; N, 0·6 per cent; P, 0·2 per cent. Examination of acidic hydrolysates showed the presence of glucose and small amounts of mannose and arabinose. The presence of glycogen was indicated by the formation of a brown colour with iodine.

The crude polysaccharide (5·9 gm.) was fractionated further by the use of cetrimide ('Cetavlon', Imperial Chemical Industries Pharmaceuticals, Ltd.) in the presence of borate[3]. Four fractions were obtained; fraction I (30 mgm.) was precipitated at *p*H 7·5, and, after acid hydrolysis, contained arabinose, glucose, mannose and amino-acids; fraction II (4·5 gm.) was precipitated at *p*H 9·5 and contained only glucose; fraction III (40 mgm.) was precipitated at *p*H 10 and contained glucose and a trace of mannose, and fraction IV (0·6 gm.) did not precipitate with cetrimide–borate and contained mainly arabinose, a smaller amount of mannose, some glucose and a trace of ribose. Fraction IV was isolated by dialysing the supernatant liquid, freeze-drying and extracting the dry solid with chloroform to remove the cetrimide.

The major fraction (fraction II) was glycogen as shown by the infra-red spectrum (peaks at 930, 845 and 758 cm.$^{-1}$), which was identical with that of an authentic sample of liver glycogen examined at the same time[4], and by the production of a brown colour with iodine. The absorption curve of the iodine complex had $\lambda_{\max.}$ 390 mμ. This was lower than that of other glycogens[5], for example, *Myletus edulis*, 420 mμ, rabbit liver, 490 mμ, and *Bacillus megaterium*, 520 mμ. The intensity of absorption was also lower (E_{390} 0·27 with 0·1 per cent glycogen in 0·01 per cent iodine and 0·2 per cent potassium iodide). The $\lambda_{\max.}$ was somewhat variable; another sample had $\lambda_{\max.}$ 405 mμ. The glycogen fraction was homogeneous when examined by boundary electrophoresis in borate buffer *p*H 9·2. The polysaccharide also migrated towards the anode in veronal buffer *p*H 9·2, but much more slowly than in borate. This may have been due to the presence of a small number of phosphate groups (phosphorus content, 0·03 per cent).

The second major fraction (fraction IV) contained 3 per cent nitrogen, which was due to the presence of protein and a small amount of nucleic acid; no amino-sugars could be detected. The fraction was heterogeneous when examined by boundary electrophoresis, containing at least two components when examined in borate, *p*H 9·2, glycine, *p*H 9·8, and veronal, *p*H 8·6. The migration in the glycine and veronal buffers could be due to the presence of protein in the fraction. Attempts to remove the protein by precipitation with trichloroacetic acid or by denaturation by shaking with chloroform–octanol (9 : 1) were unsuccessful and resulted in the production of fractions containing different proportions of protein. These results indicated that fraction IV was a mixture of polysaccharide–protein complexes.

When the organisms were grown on a medium which contained 0·4 per cent ammonium lactate instead of 5 per cent glycerol, no glycogen could be isolated from the cells, the polysaccharide being essentially similar to fraction IV. The organisms isolated after growth in 90 litres of a medium containing 'Oxoid' bacteriological peptone 1 per cent, 'Lab. Lemco' 0·5 per cent, sodium chloride 0·5 per cent and ammonium lactate 0·4 per cent for 16 days at 37° were washed free from medium and disintegrated by shaking with ballotini glass balls at 0°. The broken cells were extracted at *p*H 8, and the suspension then adjusted to *p*H 4, centrifuged at 2,600 r.p.m., and the supernatant liquid decanted. The process of extraction at *p*H 8 and reprecipitation at *p*H 4 was repeated twice on the precipitate and the three supernatant liquids were combined and treated with 3 vol. of ethanol. This material contained a considerable amount of nucleic acid which was then removed by precipitation as the lanthanum salt. The resulting polysaccharide (1·74 gm.) isolated from the non-precipitable fraction was similar to fraction IV described above in containing protein (nitrogen content, 5·2 per cent) which could not be easily removed and in being electrophoretically inhomogeneous. No glycogen could be detected by means of iodine.

This material (fraction V ; 1·7 gm.) was further separated into two components by precipitation with 50 per cent ethanol in the presence of 0·01 *M* zinc sulphate. The precipitate (fraction V*a* ; 790 mgm.) still contained protein (N, 5·0 per cent), and paper chromatographic examination of acid hydrolysates showed that mannose and glucose were present in a proportion of 5 : 1 and that arabinose was absent. The supernatant fraction also contained protein, and acid hydrolysates contained arabinose and a little mannose. This fraction was not investigated further.

Fraction V*a* had $[\alpha]_D^{20} + 20°$ in water and an infra-red spectrum which showed peaks at 975, 905, 880 and 810 cm.$^{-1}$. Further purification was carried out by the use of Fehling's solution, which is known to precipitate mannans[6]. The precipitate so produced was dissolved in dilute acid, dialysed and freeze-dried. This purified product (30 per cent by weight of fraction V*a*) contained mannose and glucose (5 : 1) and 0·96 per cent nitrogen. A positive optical rotation ($[\alpha]_D^{20} + 67°$ in water) indicated that it was probably an α-glucomannan. Examination in the ultracentrifuge (kindly carried out by Dr. D. R. Stanworth) showed the presence of three components ; one was a minor one (10 per cent) and the other two were only partly resolved even after prolonged centrifuging at 60,000 r.p.m. The infra-red spectrum in the region 1,000–800 cm.$^{-1}$ was similar to that of fraction V*a*. The spectrum had some similarities with that of a sample of yeast α-mannan (peaks at 975, 917, 885 and 813 cm.$^{-1}$) and with that of α-glucomannan II isolated from yeast cell walls by Kessler and Nickerson's method[7] (peaks at 980, 915, 890 and 815 cm.$^{-1}$) but they were not identical.

It can be concluded, therefore, that at least three intracellular polysaccharides are produced by *M. phlei*, a glycogen, an α-glucomannan and a polysaccharide containing mainly arabinose.

We thank Prof. M. Stacey for his interest and Mr. E. T. J. Chelton and Mr. C. G. James for technical assistance. Thanks are also due to the Medical Research Council for financial assistance. We are also grateful for the award to one of us (M. N.) of a scholarship under the Colombo Plan.

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Distribution of Cholinesterase within the Kidney of the Domestic Fowl

DURING investigations in this laboratory it was noticed that the distribution of cholinesterase within the fowl kidney was not uniform. The following observations are recorded in view of the sparse information regarding cholinesterases in the bird. The nomenclature of Augustinsson and Nachmansohn[1] is used throughout.

Males and females 12–14 weeks old were killed by an overdose of nembutal, the kidneys excised and frozen sections cut at 50μ and stained for true cholinesterase[2]. It was also possible to obtain paraffin sections by slightly modifying the technique ; slices 1 mm. thick were cut from the kidney and left in the substrate for 18 hr. at 37° C. The subsequent treatment was as given by Coupland and Holmes[2]. The tissue was then embedded in paraffin and sectioned in the normal way. With this procedure the very heavy deposit on the surface of the block tended to obscure a certain amount of detail, but thin sections were obtained and gave the same results as the frozen sections. In both methods pseudocholinesterase was also demonstrated by substituting butyrothiocholine iodide as substrate, and controls were obtained by preliminary incubation of the sections in 1×10^{-7} di-*iso*propylfluorophosphonate[3]. No distinction could be made between kidneys obtained from males and females.

The distribution of both true and pseudocholinesterase appeared to be very similar, but the degree of staining for the two enzymes differed considerably. Both were found in quantity in the vascular system, although this would be expected since it has been shown that cholinesterase, either the pseudo-enzyme or a mixture of true and pseudocholinesterase, is present in avian plasma[4]. The ureter wall stained very heavily for both enzymes (Fig. 1), but the tubule walls appeared to be free (Fig. 2). No cholinesterases were found in the contents of the lumen of tubules or ureters (Figs. 1, 2). Perhaps of greater importance was the heavy staining of the Bowman's capsule, particularly for true cholinesterase (Fig. 2), and this may be related to the presence of these enzymes in avian plasma. However, neither enzyme was found in the tubule or uretal contents, which suggested that

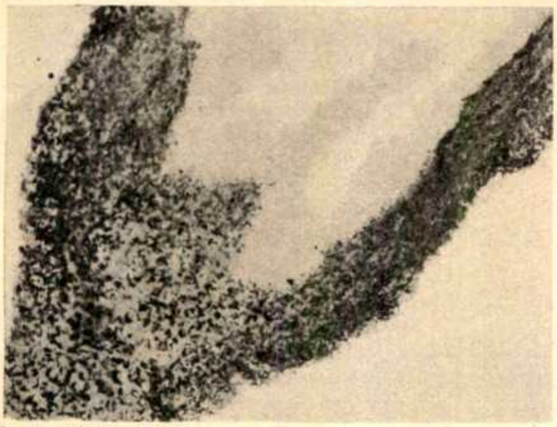

Fig. 1. Ureter. Localization of true cholinesterase only in the wall of the ureter. Frozen section

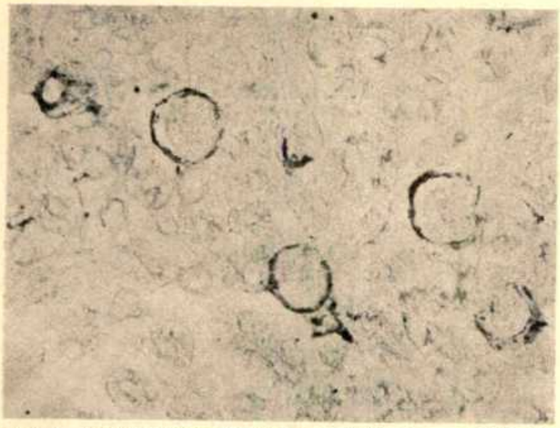

Fig. 2. Kidney section showing true cholinesterase activity in Bowman's capsule. The tubule wall and contents have not taken the stain. Paraffin section

they are not being excreted except in amounts very much lower than in plasma or in changed form.

It has been suggested that cholinesterase is involved in the sodium pump mechanism[5], and it is therefore possible that the presence of these enzymes in Bowman's capsule is related to kidney function. This suggestion must remain speculative until more is known about the physiological significance of these enzymes.

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Hæmolytic Activity of Colloidal Silica

COLLOIDAL silica is highly toxic when administered parenterally[1,2]. In isolated biological systems silica has been shown to interfere with some enzymes[3,4], activate blood clotting and pharmacologically active substances in plasma[5] and interfere with the red cell envelope[6]. Most of these effects are probably due to the adsorption and partial denaturation of proteins at the surface of silica particles[5,7].

The ultimate particle size of the colloid varies with the conditions of preparation and storage. Most of the investigators quoted have observed that aged sols have far greater biological effects than freshly prepared silicic acid solutions[2–6]. The difference is presumably due to the spontaneous growth of the particles on ageing. Using modern methods it is now possible to make silica dispersions of almost any desired particle size and thus evaluate quantitatively the differences between their biological activity. Such observations have been reported by one of us in relation to clotting of blood[8]. In the present work we have studied the effects of various types of colloidal silica on intact red cells.

To obtain silicic acid, 1–5 per cent solutions of sodium silicate were passed through a column of cation exchange resin ('Zeo-Karb 225'). According to the concentration, *p*H and subsequent heating of the material, a series of sols of particle size from 2 mμ to 6mμ were obtained (cf. Iller[9]). In addition we used commercial preparations of silica ('Ludox *SM*', Du Pont: 7 mμ and 'Syton *W*20', Monsanto: about 30 mμ).

Human red cells were washed three times with buffered isotonic saline (phosphate or bicarbonate buffer, *p*H 7·4) and suspended in saline. One-tenth volumes of serial dilutions of silica were then added to a series of tubes with the red cell suspensions and the contents were incubated at 37° C. with occasional gentle agitation. After centrifuging, the supernatant hæmoglobin was measured and expressed as a percentage of the total.

Fig. 1 shows the result of a typical experiment with a 2 per cent cell suspension incubated with silica for 30 min. It is obvious that colloidal silica can be a very potent hæmolytic agent. For example, 1 μgm./ml. of the 30-mμ particles lyses about 50 per cent of the cells. Since the surface area of this silica is somewhat less than 1 cm.^{2}/μgm. while that of 2 per cent cell suspension is about 150 cm.^{2}/ml., it follows that contact of silica with a very small portion of the red cell surface is sufficient to produce hæmolysis. The hæmolytic activity decreases with the particle size

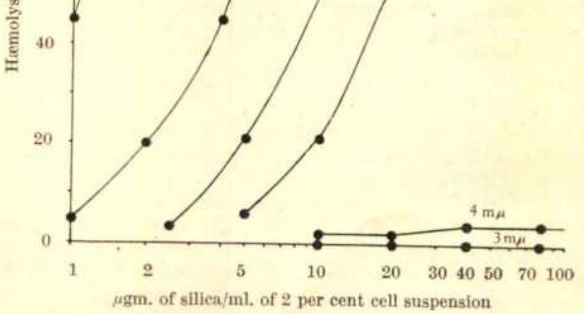

Fig. 1. Hæmolysis of 2 per cent red cell suspension incubated with colloidal silica for 30 min. at 37° C. The figures above the curves indicate the mean particle diameter of the colloid

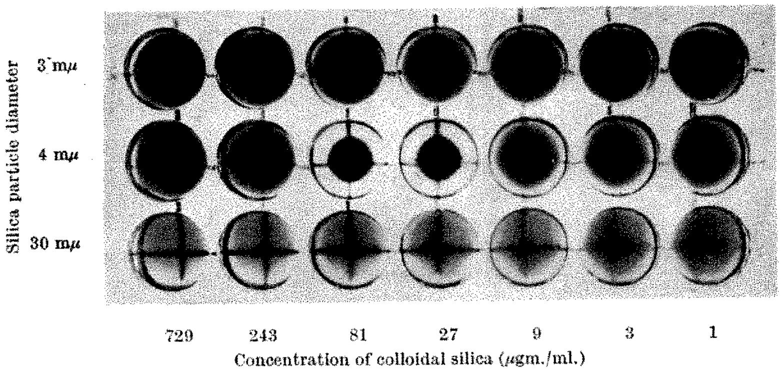

Fig. 2. Hæmagglutination plate viewed from above. 2 per cent red cell suspension was incubated with colloidal silica. Black lines were drawn on the reverse side to emphasize the transparency of the hæmolysed samples. Top row, no visible change; second row, zonal agglutination; bottom row. hæmolysis

of the colloid until it becomes negligible at about 4 mμ diameter. We have also observed that particles in the range of 3·5–7 mμ have a powerful agglutinating effect on the red cells, with a well-marked prozone (Fig. 2). Silica of 3-mμ or smaller particle size has no such activity but protects the cells against the agglutination and hæmolysis by the larger particles. This 'blocking' effect occurs only when the smaller particles are introduced into the cell suspension before the larger ones and it persists after repeated washing with saline, which probably indicates an irreversible adsorption of silica on to the erythrocyte surface. Such 'coated' red cells have other properties in addition to their resistance to the larger particles of silica. Thus they are not hæmolysed by streptolysin *O* and the saponin fragility is somewhat decreased. On the other hand, these cells become more susceptible to immune (rat) hæmolysins and to primaquine. The osmotic fragility is not altered, nor is there any obvious abnormality in appearance except that the cells do not become spherocytic when examined between glass slides and coverslips as do normal washed erythrocytes[10].

In all these experiments it is essential to use well-washed cells because even small traces of plasma markedly inhibit the action of silica. The addition of 1 : 200 plasma to the system as in Fig. 1 completely prevents hæmolysis. The plasma must, however, come in contact with silica before the latter is adsorbed by the red cells. Even a few seconds delay suffices to abolish the protective effect of the plasma. This phenomenon is evidently due to the coating of the silica by certain plasma components. The substances in question are non-dialysable and can be recovered in alkaline eluates from powdered silica which has been exposed to plasma or serum. Such eluates retain their activity after heating to 100° C.

Our observations on the agglutinating and blocking activity of silica agree with the qualitative findings of Renton and Hancock[6], who suggested that these effects may be analogous to those of agglutinating and incomplete antibodies, respectively. The present results extend this model to cover hæmolysis as well.

Although our present knowledge of the red cell structure is still fragmentary, the reaction between silica and the cell membrane may be visualized in general terms as a two-stage process: first, a rapid adsorption of the particles by the cell surface, due mainly to the ionic attraction between silica and the amino-groups at the cell surface and further reinforced by hydrogen bonds and van der Waals forces[7]; secondly, protein denaturation, the extent of which may depend purely on the geometrical relation between the colloidal particles and the protein molecules, as was previously suggested in connexion with blood clotting[5,8]. Individual silica particles of less than 3·5 mμ may not provide sufficient contiguous surface to permit significant unfolding of the much larger protein molecules. Contact with larger particles will, however, allow such unfolding (denaturation), and the resulting interference with the integrity of the cell membrane then leads to agglutination or hæmolysis according to the degree of denaturation. The geometrical picture may, of course, be over-simplified and other physical differences between the large and the small colloidal units may be contributory factors[11]. Despite these reservations, colloidal silica may prove a useful tool in the elucidation of the red cell structure.

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PHYSIOLOGY

The Psychotomimetic Properties of 3,4,5-Trimethoxyamphetamine

dl-3,4,5-TRIMETHOXYAMPHETAMINE (*dl*-TMA), first prepared as a homologue of mescaline in 1947 [1], has remained to a large measure unexplored. A single paper has described its effects in human subjects[2], demonstrating that the drug allows stroboscope-induced hallucinations at the levels employed, namely, 0·8–2·0 mgm./kgm., orally. In the present work, *dl*-3,4,5-trimethoxyamphetamine was synthesized by the general method of Ramirez and Burger[3] and was chemically identical to that previously described[1]. Pharmacological similarity was demonstrated by the administration of between 1·6 and 2·0 mgm./kgm. as the hydrochloride, to three adult male subjects. The responses were found to be parallel in intensity and duration to those described earlier[2], although the vivid hallucinations reported were not observed.

It has been found, however, that with an increased dosage of the drug, the psychotropic response changed in a most dramatic manner. Dosages of 2·8–3·5 mgm./kgm. were given to five adult male subjects, all of whom had had previous experience with either mescaline or lysergic acid diethylamide.

The physical changes observed were similar for all subjects employed. In each case, after about half an hour, there ensued a period of autonomic distress, characterized by sweating, tremor, and chills as well as nausea and dizziness. This phase lasted no more than an hour. The slight but definite systolic blood pressure increase of about 10 mm. mercury returned to normal at the end of this phase. The concurrent diastolic increase was barely perceptible (*c.* 2 mm. mercury). During the remainder of each experiment (approximately another 6–8 hr., which includes the period of extreme mental derangement described below) very few overt physical signs of drug effect were evident. Pupillary dilatation and slight motor inco-ordination were noted. Pulse increase was negligible.

The psychic changes observed, however, were extreme and showed considerable individual variability. The initial effects, at about 2 hr. from the ingestion of the drug, were mescaline-like, involving intensification of visual experience, including amplification and distortion of colour, texture, form, and spatial relationships. These effects were distinctly less than those expected from a pharmacologically equivalent dose (2 ×) of mescaline. Auditory and tactile sensations were also intensified, and both paræsthesia and synæsthesia were noted on occasion. None of the subjects displayed the enhanced capacity for empathy characteristic of mescaline.

The emotional responses elicited during the period of maximum *dl*-3,4,5-trimethoxyamphetamine intoxication (3–5 hr. from the start of the experiment) were striking in their intensity. Anger, hostility, and megalomaniac euphoria dominated the subject's thoughts and conversation. Actual acts of hostility were not observed, but it was felt that, in at least two subjects, provocation would have precipitated homicidal violence. All subjects reported imagery, either patterned or scenic, with eyes closed. Recollection of past experiences did not seem to be enhanced, and intellectual performance appeared to be somewhat impaired. Once the plateau of intoxication had passed, return to normal was rapid. The subjects experienced no clouding of consciousness, and subsequent recall of events was excellent.

Due to the unexpectedly anti-social character of the response to larger doses of the drug (a response unobserved in more than 40 mescaline subjects), a warning concerning adequate supervision during experimentation with this new drug seems desirable.

Investigations employing the optical isomers, as well as structural homologues, of *dl*-3,4,5-trimethoxyamphetamine are in progress.

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The Conversion Ratio as a Discriminatory Test for Thyroid Activity in Fish

THE rate of conversion of administered inorganic radioiodine into protein-bound radioiodine of the plasma has been used frequently to evaluate thyroid activity in mammals. The test is recognized as having good discriminatory power in higher vertebrates[1,2], but the relatively large sample of blood required for separation of serum by the usual methods has made the test impractical for small fish. A method is described here for determining protein-bound radioiodine as an *in vivo* test in fish of 30 gm. or more and as a terminal test in fish as small as 1 gm.

Each fish received an intraperitoneal injection of carrier-free iodine-131, the dosage depending on the weight of the fish and the detection instrument used. When using a scintillation well-counter, the instrument of choice, 0·1 μc. iodine-131 per gm. body-weight was sufficient. Blood sampling for *in vivo* determinations in anæsthetized trout were carried out by direct cardiac puncture with a ¼ c.c. heparinized syringe. Blood was transferred to a heparinized capillary tube and the vacant end of the tube plugged with 'Plasticene' or sealed with a small concentrated flame such as that of a propane torch. Blood may be obtained from very small fish by cutting off the tail and collecting the blood as it flows from the caudal artery into a heparinized capillary tube. Sealed tubes were centrifuged in a hæmatocrit centrifuge or in a 15-ml. bucket centrifuge fitted with hollow wooden adapters for the tubes. After separation of the cells, the tubes were scratched and broken between cells and plasma and the plasma was expressed into a 15-ml. centrifuge tube containing 2 ml. of 20 per cent trichloroacetic acid. The protein precipitate was stirred vigorously with a glass rod and centrifuged down. The supernatant containing the inorganic iodine-131 was decanted and saved. The precipitate was washed three times with 2-ml. portions of 2·5 per cent trichloroacetic acid, each time breaking up and stirring the precipitate and then centrifuging. All trichloroacetic acid washes, totalling 8 ml., were combined and saved. The precipitate was then dissolved in sodium hydroxide and diluted to the same volume as the combined supernatant washings. Finally, the radioactivity of both fractions was counted. Using the well-crystal scintillation counter, aliquots of supernatant and dissolved precipitate were pipetted into counter tubes and counted to 10,000 counts. An end-probe scintillation counter was occasionally used, and greatest sensitivity was achieved by securing the probe with crystal facing up in a lead castle and placing the fractions contained in a small beaker directly on the crystal. After subtraction of the background count, percentage of protein-bound iodine-131 is calculated as :

$$\frac{\text{Prot.-bound iodine-131 in counts per min. (precip.)}}{\text{Prot.-bound iodine-131 + iodine-131 (combined counts of precip. and supernat.)}} \times 100$$

Serial determinations in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*) have shown that protein-bound radiothyroxine in the plasma gradually increases relative to inorganic radioiodide until approximately the fourth or fifth day, after which it begins to fall. The rate of increase usually is most rapid just before the inflexion and represents the period when the greatest percentage

Table 1.' COMPARISON OF THYROID RADIOIODIDE UPTAKES AND CONVERSION RATIOS IN NORMAL AND THIOUREA-TREATED FLOUNDER

Controls		Thiourea-treated	
4-day uptake	4-day conversion ratio	4-day uptake	4-day conversion ratio
6·40	14·3	2 05	1·10
2·23	18·3	2 80	0·22
5 08	10·9	5 00	0 19
6 90	16 0	2·16	0 07
8·90	13·8	2·93	0·54
		2·05	0·42

of labelled thyroxine molecules is secreted from the thyroid gland. When breakdown or excretion of radiothyroxine exceeds its rate of appearance in the blood, the conversion ratio falls.

Usually only one determination is made at a fixed interval of time after the injection of radioiodide. Sampling should be made before the maximum value for protein-bound iodine-131 is reached, and the ideal time to sample will vary with the species and with the experimental conditions. In general, the more active the thyroid gland, the earlier the ideal sampling time will be. A 4-day interval between administration of the tracer dose and sampling has been found satisfactory for rainbow trout and starry flounder under normal conditions. Periods longer than 4 days rarely prove worth while, because the reliability of the method is rapidly limited by loss of radioiodide through excretion and physical decay.

In Table 1 the 4-day conversion ratios and the 4-day thyroid radioiodide uptakes of normal starry flounder and flounder immersed 11 days in 0·04 per cent thiourea are compared. In this experiment, the 4-day conversion ratio is of far greater value than thyroid radioiodide uptake for determining the degree of thyroid inhibition achieved. The conversion ratios average 14·6 for the normal flounder as compared with 0·42 in those flounders treated with thiourea; this shows that blockage of hormone synthesis is essentially complete. Thyroid radioiodide uptake is comparatively useless for such a test, because some radioiodide is accumulated by the inhibited gland.

It is desirable to reduce the time-interval before sampling if the experimental treatment is expected to stimulate thyroid activity greatly. Table 2 shows the 2-day conversion ratios of starry flounder treated with thyroid-stimulating hormone. Two injections of the hormone were given, one three days and the other six days before the injection of radioiodide for the determination of protein-bound iodine-131.

In spite of the small size of sample, the relative effectiveness of the different dosages of thyroid-stimulating hormone is easily estimated.

Many factors influence the conversion ratio, making its mathematical analysis exceedingly complex[3].

Table 2. EFFECT OF THYROID-STIMULATING HORMONE ON THE 2-DAY CONVERSION RATIO OF STARRY FLOUNDER (*Platichthys stellatus*)
Experimental temperature, 14·0° C.; experimental salinity, 26 per mille

Controls		Treated with thyroid-stimulating hormone					
		0 0001 U.S.P. units/gm.		0·001 U.S.P. units/gm.		0·01 U.S.P. units/gm.	
Body-weight	2-day con-version ratio	Body-weight	2-day con-version ratio	Body-weight	2-day con-version ratio	Body-weight	2-day con-version ratio
126 5	1 96	97 0	4·5	84	39·7	79·5	38·0
93·5	1·25	93 5	2 0	77·5	13·8	76	46·3
81 0	1·94	71 5	13 7	75	22·6	68 5	37·2
	av. 1 72		av. 6·7		av. 25·4		av. 40·5

However, for purposes of distinguishing different levels of thyroid activity, the test is accurate, simple to perform and possesses greater discriminatory power than the uptake of radioiodide by the thyroid gland. It has the important advantage of measuring the relative rate of secretion of thyroid hormone rather than the more indirect iodide-trapping activity of the thyroid gland. The test should prove particularly valuable in assessing thyroid activity of very small fish.

Thyroid-stimulating hormone was kindly supplied by Wayne Donaldson, of Parke, Davis and Company, Detroit. Financial assistance was received through grant *G*-8502 of the National Science Foundation, Washington, D.C., awarded to W. S. Hoar, University of British Columbia.

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Transport of Phosphate into the Erythrocyte

THE active transport of inorganic phosphate into the red blood cell is generally believed to be closely related to glycolysis, although the actual mechanism of transfer is unknown[1]. Gourley[2] considered that phosphate enters the erythrocyte through the formation of adenosine triphosphate at the cell surface. Prankerd *et al.*[3] and Bartlett[4] concluded from their investigations that inorganic phosphate is transferred into the cell by incorporation into 1,3-diphosphoglyceric acid. Our own experimental evidence suggests that the transfer of inorganic phosphate is independent of glycolysis.

Whole blood was incubated at 37° C. with orthophosphate labelled with phosphorus-32. The disappearance of phosphorus-32 from the plasma was measured over a 2-hr. period and calculated on the basis of a 40 per cent hæmatocrit. In a series of eight samples the rate of disappearance of phosphorus-32 in the presence of glucose (200 mgm. per cent) was 29·3 per cent/hr. ± 3·1 per cent. When iodoacetic acid was included in the incubation mixture to a final concentration of 10^{-3} molar, lactic acid production was completely inhibited, and phosphorus-32 left the plasma in nine samples at a mean rate of 12·5 per cent/hr. ± 1·1 per cent. Eight samples of whole blood incubated with glucose, iodoacetic acid and adenosine (6·25 m.mol./l.) were found to show a rate of disappearance of plasma phosphorus-32 similar to those samples incubated with glucose alone (29·8 per cent/hr. ± 3·7 per cent). Under these conditions there was again no production of lactic acid. The similarity of the two rates of influx with glucose alone, and with glucose, iodoacetic-acid and adenosine suggests that the mechanism of transfer of phosphate into the cell under these two conditions may be identical. To establish further the similarity of transport in the presence or absence of glycolysis we studied the effects of variation of incubation temperature and of external phosphate concentration on the rate of entry of phosphate into the erythrocyte.

In Fig. 1, it will be noted that as the external phosphate concentration is increased there is an increase in the rate of entry of orthophosphate into the red cell. This increase is identical in the presence of either glucose, or glucose, adenosine and iodoacetic acid. In Fig. 2, the effect of change of temperature is shown. An increase in temperature causes an increase in the transfer-rate constant which is identical under both conditions. These findings indicate that phosphate enters the red blood cells at similar rates whether glycolysis is proceeding or not.

To find out whether the phosphate which entered the cell under these two conditions would be metabolized in a similar fashion the distribution of radioactive phosphorus-32 within red cell phosphate esters was studied by the method of anion exchange column chromatography of Bartlett[3]. It may be seen in Fig. 3 that in the presence of glucose most of the phosphorus-32 was found in adenosine triphosphate (ATP) and 2,3-diphosphoglyceric acid (2–3 DPG) (Fig. 3, *A*). Inhibition of glycolysis by iodoacetic acid (Fig. 3, *B*) prevented the entry of ^{32}P-orthophosphate into these esters. When adenosine was included radioactivity was found in the 0·1 *N* ammonium chloride fraction, with very little in adenosine triphosphate (ATP) or 2,3-diphosphoglyceric acid (2–3 DPG) (Fig. 3, *C*). In *A* and *B* the amount of organic phosphate in the 0·1 *N* ammonium chloride fraction (known to contain hexose diphosphates but no monophosphate[5]) was low. In *C*, however, this fraction contained greater quantities both of organic phosphate and fructose. Fructose was determined by the Roe modification of the Seliwanoff procedure[6]. In two experiments it was found that the excess phosphate and fructose in *C* occurred in the ratio of 1·83 moles of phosphorus/mole of fructose. These findings suggested that the radioactivity which appeared in the 0·1 *N* ammonium chloride fraction under these conditions was in fructose-1,6-diphosphate.

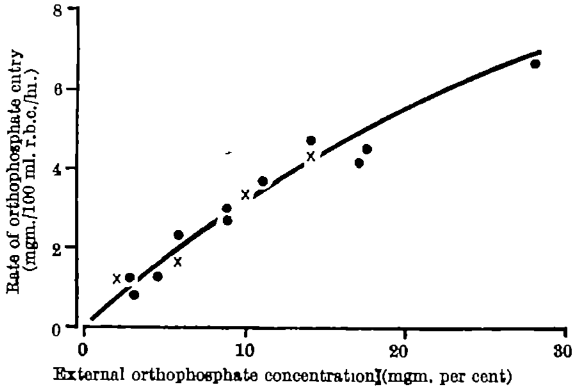

Fig. 1. Effect of variation of external phosphate concentration on the rate of total phosphate entry into the red blood cell. Washed erythrocytes were suspended in veronal acetate buffer (pH 7·45); and incubated for 2 hr. at 37° C. with 0 2 μc. phosphorus-32 sodium phosphate in the presence of: (1) glucose 200 mgm. per cent (●); (2) glucose 200 mgm. per cent, iodoacetic acid 10^{-3} *M* and adenosine 6 25 m*M* (×)

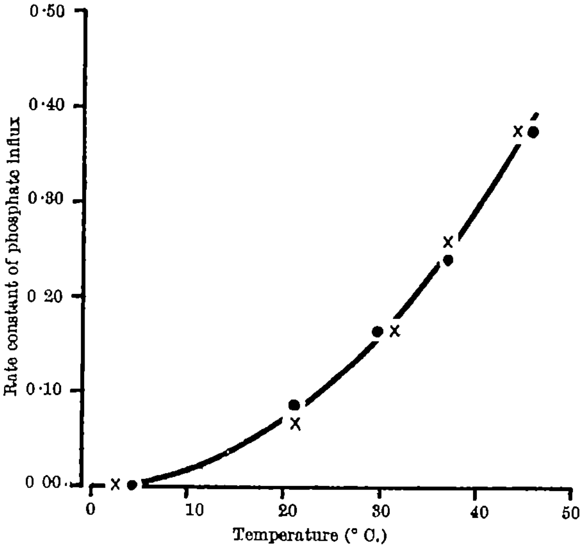

Fig. 2. Effect of variation of incubation temperature on the rate constant of phosphate influx into red blood cells. Washed erythrocytes were suspended in veronal acetate buffer (pH 7 45, inorganic phosphate concentration 8 0 mgm. per cent). These cells were incubated for 2 hr. at 37° C. with 0·2 μc. phosphorus-32 sodium phosphate in the presence of: (1) glucose 200 mgm. per cent (●); (2) glucose 200 mgm. per cent, iodoacetic acid 10^{-3} *M* and adenosine 6·25 m*M* (×).

Rate constant of phosphate influx in each experiment was corrected to a hæmatocrit of 40 per cent

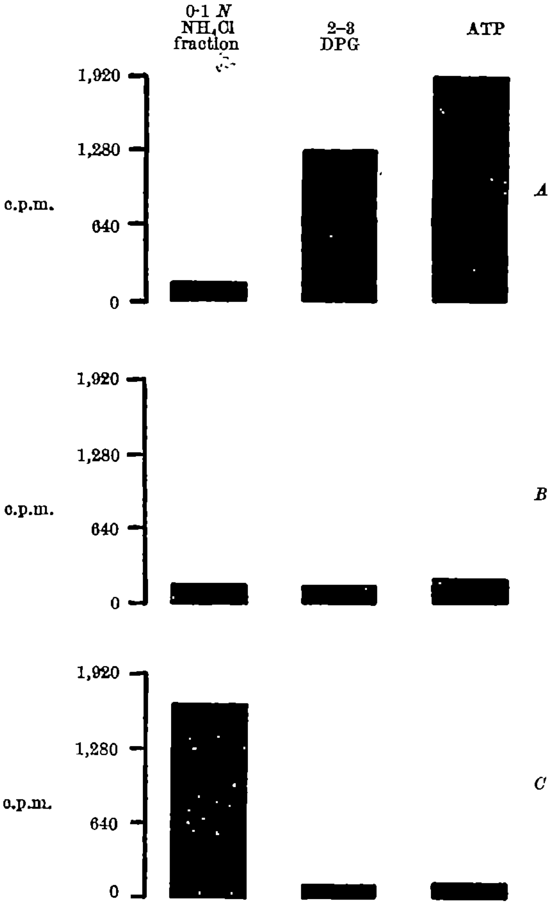

Fig. 3. The radioactivity (c.p.m.) of erythrocyte phosphate esters after 2-hr. incubation at 37° C. (20 ml. of blood with 20 μc. of phosphorus-32) in the presence of: *A*, glucose (200 mgm. per cent); *B*, glucose (200 mgm. per cent) and iodoacetic acid 10^{-3} *M*; *C*, glucose (200 mgm. per cent), iodoacetic acid 10^{-3} *M* and adenosine 6·25 m*M*

In the presence of glucose alone (Fig. 3, *A*) orthophosphate is probably esterified by way of the glyceraldehyde-3-phosphate dehydrogenase reaction. With glucose, iodoacetic acid and adenosine, glycolysis is inhibited and inorganic phosphate is esterified, presumably in the purine nucleoside phosphorylase reaction, to form ribose-1-phosphate. This material can then be metabolized through the hexose monophosphate shunt and result in an accumulation of fructose-1,6-diphosphate[7]. Under these two conditions inorganic phosphate enters the red blood cell at the same rate yet follows different metabolic pathways. The identical kinetics of phosphate transfer, whether glycolysis is active or inhibited, suggests that the limiting step of entry of phosphate into the red blood cell is similar under both conditions and is not dependent on glycolysis.

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Control of Fertility by 6-Azauridine

We have previously suggested that agents known to inhibit cell division might be effective ovicides in mammals, in amounts which are non-toxic to the pregnant female[1]. We have reported on a few such agents, and our results in general supported the hypothesis that it might be possible to separate the effects on the ovum and on the maternal organism[2]. Since that time, we have examined a number of other substances, and in this communication we wish to direct attention to one of these in particular. This substance, 6-azauridine (2-ribofuranosyl-*as*-triazine-3,5(2*H*,4*H*)-dione) ('AzUR'), is so far the one which most closely approaches our aim of finding an oral agent which might control human pregnancy, with minimal toxic effects.

'AzUR' has been shown to be a highly active inhibitor of the growth of some species of bacteria[3], and of mammalian tumours, both in tissue culture[4,5] and *in vivo*[4,6]. It has also been found to be effective in leukæmia in man[7]. It is reported to have very low toxicity in mice[4,6,8] and in man[7,8], though it is somewhat toxic in dogs[4,8].

In our experiments, 'AzUR' was dissolved in water and given to mice by stomach tube in doses related to the weight of the animals. As in our previous work, we used Swiss albino mice. It has been reported that 'AzUR', because of its rapid rate of excretion, is more effective when given in divided doses[4]. In some of our experiments, therefore, we compared the effect of administering the substance in one single dose with that of administering it in three equal fractions of the daily dose at intervals of 8 hr.

Toxicity. The mildest sign of toxicity was a temporary fall in weight, which was rapidly regained when the 'AzUR' was withdrawn. More severe toxic effects were diarrhœa, a staring coat and death.

In non-pregnant animals, we have found that single doses of 0·5 mgm./gm., given for 9 consecutive days, were without effect in mature animals. In weanling animals, the same dose produced a significant but temporary loss in weight. Neither the younger nor the older mice showed any impairment of fertility when they were afterwards mated, and they produced normal litters. Thus, 'AzUR' does not appear to affect unfertilized ova.

In pregnant animals, the occurrence of toxicity depended on the size of the daily dose of 'AzUR', the number of days during which it was given, whether it was given as a single dose or in divided doses, and the stage of pregnancy. A single dose of 4 mgm./gm., given on the fourth day of pregnancy, was without toxic effect. Three consecutive single doses given on the fourth, fifth and sixth days killed some animals at 4 mgm./gm., and caused loss of weight at 1 mgm./gm.

Because of these effects, most of our further work was carried out with doses of 0·5 mgm./gm. Single or divided doses at this level, given for 1–4 days, were non-toxic in the early stages of pregnancy. Given for three consecutive days on or after the tenth day of pregnancy, they led to the death of several of the mothers. We believe that this was secondary to the death of the large conceptus at these late stages of pregnancy (see below).

Effect on pregnancy. The effect on pregnancy, like the toxicity, also depended on the dose, the duration of administration, the state of pregnancy and whether the drug was given in one dose or in divided amounts.

The most regularly effective time of dosing was for three consecutive days from the second, third or fourth day of pregnancy. Thus, in single doses over three days from the fourth to sixth day of pregnancy, 1 mgm./gm. interrupted every pregnancy, whereas 0·5 mgm./gm. interrupted about 50 per cent of pregnancies. Given for four days (third to sixth) instead of three days, single doses of 0·5 mgm./gm. interrupted all pregnancies. In divided doses, however, 0·5 mgm./gm. a day was invariably effective in interrupting pregnancy when given for only three days.

'AzUR' was less effective in the very earliest stages of pregnancy, so that 0·5 mgm./gm. given daily in divided doses for the first three days after copulation interrupted only 9 out of 16 pregnancies. Again, the same dose of 'AzUR' interrupted only half the pregnancies when given from the tenth to twelfth day, and none when given from the fourteenth to sixteenth day. There was, however, an important difference in the litters born to mothers dosed early, or late, in pregnancy. Those born to mothers dosed early in pregnancy survived, and were apparently quite normal; they were able, for example, themselves to conceive and rear their own young. Those born to mothers dosed late in pregnancy were smaller in number, and most of the young died within two days of birth.

The time of termination of pregnancy was related to the dosing schedule. Thus, with single doses over four consecutive days, from the third to the sixth day, pregnancy was terminated between the ninth and fifteenth day with 1 mgm./gm., and between the fourteenth and eighteenth day with 0·5 mgm./gm. Fractional doses totalling 0·5 mgm./gm. daily produced even earlier termination, between the eighth and the ninth day.

'AzUR' thus appears to have a selective action upon the conceptus during part of its existence. At particular levels, it does not affect the mother. Nor does it exert its maximum ovicidal effect during the very earliest stages of pregnancy, though this may be due to inaccessibility of the ovum rather than to its insensitivity. Again, it gradually ceases to affect the conceptus during the later stages of pregnancy, until eventually it allows the birth of some live young. Nevertheless, the size of litter is reduced, and this, together with the absence of toxicity on the mother at other stages, makes us believe that the apparent toxicity in the late stages of pregnancy are due to the death of one or two fœtuses, which by then are quite large.

A great deal more work needs to be done with 'AzUR' before we can decide whether it might be of use in the control of human fertility. There are, however, already several features of the incomplete results so far obtained which provide a considerable incentive to pursue this work. First, at doses which interrupt pregnancy, 'AzUR' has no effect on the unfertilized ovum, nor—with one exception—on the mother. The exception is the ill-effects produced when 'AzUR' is given late in pregnancy. Secondly, young born from mothers which were inadequately dosed seem to be perfectly healthy, and are themselves capable in due course of producing and rearing normal young. This lessens the possibility that, if 'AzUR' were to be used in the control of human fertility, congenital malformations might be produced in young born through inadequate dosing. Thirdly, there is now considerable evidence that 'AzUR' has very low toxicity in man. Amounts equivalent to 0·3 mgm./gm. daily, given intravenously at intervals of 8 hr. over as long as four weeks, are said to have no toxic effect whatever[7,8].

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L-Glutamine and the Growth of Embryonic Chick Tibiotarsi *In vitro*

IN 1955 [1] Kieny published an account of the amino-acid requirements of eight-day embryonic chick tibiæ cultivated on various simple mixtures of inorganic salts and amino-acids, including L-glutamic acid. She reported that although the addition of glutamine to the media caused some increase in the length of the bones they also lost weight. In a later paper[2], however, she concluded that the substance had no effect on the bones. Recently, Biggers, Gwatkin and Heyner[3] have described the growth of embryonic chick, rat and mouse tibiæ on a relatively simple medium (denoted *BGJb*), which does not contain L-glutamic acid. It has been demonstrated in simple total deficiency experiments that L-glutamine is essential for growth in all three species, whether the response is measured in terms of length, wet weight or dry weight.

Other workers[4] have shown recently that explants of chick-heart tissue do not require L-glutamine because they contain glutamine synthetase. This enzyme catalyses the irreversible reaction:

$$\text{L-glutamic acid} + \text{adenosine triphosphate} + \text{ammonia} \rightarrow \text{L-glutamine} + \text{adenosine diphosphate} + \text{phosphate}$$

If we assume that glutamine synthetase is present in embryonic chick bones, then it is possible to argue that sufficient L-glutamine was synthesized from L-glutamic acid in Kieny's experiments to produce a maximal response. If so, then the addition of more L-glutamine to the system would produce no further effect. One can also argue that it is likely that some L-glutamine was formed to a minor extent in our experiments[3] since L-glutamic acid can arise from glucose in embryonic chick bones[5].

In order to settle these questions, the responses of seven-day embryonic chick tibiotarsi to different concentrations of L-glutamine have been studied in the presence and absence of L-glutamic acid. A 2 × 3 factorial arrangement has been employed: 1, 5 and 25 mgm. per 100 ml. of L-glutamine, and 0 and 25 mgm. per 100 ml. of L-glutamic acid, incorporated in all six treatment combinations in medium *BGJb*. In a preliminary experiment the concentration-response line of L-glutamine was determined. The results showed that the response becomes maximal in the range of 8·75–17·5 mgm. per 100 ml. Thus the concentration of 25 mgm. per 100 ml. which has been employed for the upper level of L-glutamine in the factorial experiment is more than sufficient to elicit a maximum response.

The uniformity of the paired limbs from embryos has been exploited by comparing the effects of the six treatments in a balanced incomplete block design. The pairs of tibiotarsi from fifteen embryos were allotted at random to the fifteen combinations between the six treatments taking two at a time. The bones were cultivated, using the floating lens paper technique, at 37° C. for 6 days in an atmosphere of humidified 5 per cent carbon dioxide in air. The medium was renewed every second day. At the end of the experiment the length of the bones was determined. The analysis of variance was done after converting all the data to logarithms. Only the intra-block error was estimated, and the adjusted treatment sum of squares were partitioned using the methods described by Kramer and Bradley[6].

The adjusted means are shown in Table 1, together with the analysis of variance. It is obvious that there is a highly significant linear regression of log length on the log concentration of L-glutamine in the medium and that some response occurred with only 1 mgm. per ml. L-glutamine in the medium. Since the L-glutamic acid and the interaction mean squares are not significant, there is no evidence that L-glutamic acid affects the response in length of the bones to L-glutamine. Thus there is no evidence that L-gluta-

Table 1. THE ADJUSTED MEAN LOG LENGTHS (MM.) OF SEVEN-DAY EMBRYONIC CHICK TIBIOTARSI AFTER CULTIVATION FOR 6 DAYS ON MEDIUM *BGJb*, IN THE PRESENCE OF VARIOUS COMBINATIONS OF L-GLUTAMINE AND L-GLUTAMIC ACID

L-Glutamic acid (mgm. %)	L-Glutamine (mgm. %) 1	5	25
0	0·370	0·444	0·539
25	0·394	0·429	0·579

Mean initial log length = 0·205.

Analysis of variance

Source of variation	D.F.	Mean square
Between embryos (unadj.)	14	0·0098
Between treatments (adj.)	(5)	
L-Glutamic acid	1	0·0008
L-Glutamine:		
Linear	1	0·0893*
Quadratic	1	0·0053
Interaction	2	0·0023
Intra-block error	10	0·0032

* $P < 0·001$

mic acid is a precursor of L-glutamine in embryonic chick tibiotarsi, for if synthesis of L-glutamine did occur, the two concentration-response lines would be expected to separate, particularly at the lower levels of L-glutamine. Whether this is because this tissue does not contain glutamine synthetase remains to be determined.

The results also indicate why Kieny failed to demonstrate an effect of glutamine. The concentration used by her was only 0·16 mgm. per 100 ml., which is right at the lower end of the concentration-response line.

Hence, it is clear that L-glutamine is essential for the elongation of embryonic chick tibiotarsi *in vitro*, and that it is unlikely that it is produced from L-glutamic acid by this tissue. The importance of this compound to the cartilaginous rudiment is scarcely surprising in view of its essential role in the synthesis of purines[7] and chondroitin sulphate[8].

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Organization of Function in Trypanosome Flagella

SOME cilia and flagella beat in a complex three-dimensional manner, and, in addition, the angular velocity at the tip is greater than at the base. Presumably the bundle of fibrils which make up a cilium or flagellum contains both conducting and contractile elements[1]. Trypanosome flagella, in common with most flagella and cilia, have 9 peripheral and 2 central fibres[2].

Acridine derivatives are well known to produce photosensitivity in cells and tissues. In the presence of pure 2 : 8-diamino-N-methylacridinium chloride (acriflavine) trypanosomes respond to light of wavelength 430–465 mμ first by an increased motility, secondly, by a stiffening of the flagellum which soon stops moving, and thirdly, by loss of refractility of the whole organism. To localize the site of photosensitivity a system of illumination was organized so that there was a circle of bright light about 4μ diameter and a background illumination of subdued red light to enable the operator to see a trypanosome throughout exposure without causing any additional damage.

The apparatus consisted of a microscope set up for Köhler illumination using a 6-V. 30-watt tungsten lamp. A ×40 objective (N.A. = 0·70) was used in place of the standard substage condenser and the trypanosomes were viewed through a ×80 oil immersion objective. An additional diaphragm held in a micromanipulator was placed close to the lamp house. This diaphragm consisted of an unprotected deep red gelatine filter (Wratten No. 70) which was carefully pierced with a pin from either side. The hole measured about 400μ in diameter. The image of this hole was formed in the plane of the object and was 4μ in diameter. A small circle of the same gelatine filter inserted into the eyepiece enabled the operator to observe both the trypanosome and the circle of light throughout an experiment.

Trypanosoma gambiense (an old laboratory strain) and *T. rhodesiense* (a newly isolated strain) were used in this experiment. The organisms were obtained from rat blood by centrifugation and diluted with a buffered-glucose Ringer's solution (pH 7·2) to give a count of about 12,000/μl. The final concentration of acriflavine was $10^{-5·1}$ M for *T. gambiense* and $10^{-4·81}$ M for *T. rhodesiense*; these concentrations were known from previous experiments to give complete immobilization in only a few seconds when the whole organism was illuminated. The movement of *T. rhodesiense* was reduced by the addition of 25 per cent of dextràn powder (Benger, Ltd.) to the diluting medium. The temperature of incubation and exposure was about 20° C. No difference in behaviour was observed between the two species of trypanosome. The combined results are expressed in Table 1.

The circle of light was localized in one or more of the four regions A, B, C and D as indicated in Fig. 1.

When the circle of light was brought to bear on a trypanosome, increased motility was first observed. Continued exposure of any part of the flagellum produced a slowing of that part and movement usually ceased after 30–40 sec. (see Table 1). Exposure

Table 1. DETAILS OF THE RESPONSE OF TRYPANOSOMES WHEN REGIONS OF THE CELL ARE EXPOSED TO BRIGHT LIGHT

Exp.	Region exposed	Time of exposure (min.)	No. of tryps. exposed	No. of tryps. responding	Response as regional motility *A*	*B*	*C*	*D*
1	*A*	<2	11	10	−	+	+	+
2	*A*	2–3	5	5	−	−	−	−
3	*B*	1	9	6	+	−	+	+
				2	−	−	+	+
4	(*C*+*D*)	1	13	12	+	+	±	−
5	(*C*+*D*)	>3	5	5	+	+	−	−
6	*D*	c. 1	3	3	+	−	+	−
	B	c. 1						
7	(*C*+*D*)	c. 1	4	3	−	+	±	−
	A	c. 1		1	−	−	−	−

Regional motility: − stationary; + passing waves, ± variable response or doubtful observation.

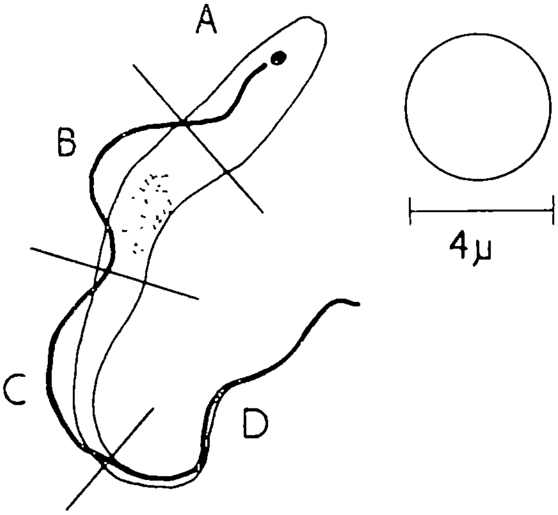

Fig. 1. Regions *A*, *B*, *C* and *D* of a trypanosome which were exposed to bright light. The circle, representing the area illuminated, is drawn to the same scale

for 1 min. of region *A* caused little obvious effect. Close examination after exposure revealed that the flagellum in this region neither accepted nor initiated any waves of contraction as it had done previously. Further exposure of region *A* caused sudden cessation of all movement of the flagellum and rapid loss of refractility of the organism. The time from full motility to this apparent state of death was less than 15 sec. in all cases. The total time taken for exposure of the region *A* to cause death was between 2 and 3 min. As the cone of light coming to a focus made an angle of 28° to the optic axis these longer exposures might have caused damage to other parts of the trypanosomes moving in and out of the cones of light above and below the focus. Exposure of the flagellum ($C + D$, group 5) for more than 3 min. showed that the action was specific to region *A*.

These findings can be summarized: In the presence of acriflavine, light on any part of the flagellum causes that part to stop moving, while the remainder of the flagellum continues to undulate. A similar quantity of light has no observable effect on the kinetoplast, but prolonged exposure of the kinetoplast results in death of the trypanosome.

As a rule, locomotion of trypanosomes occurs with the flagellum leading. A conducting element is therefore postulated to initiate waves of contraction that pass from the tip of the flagellum to the cell body. Groups 3 and 6 show that the flagellum behaves as though either a conducting element functions through the region of an injured contractile element or that contractions have local and self-initiated origins. In view of the cessation of all movement after prolonged exposure of the kinetoplast and of the complex screw-like waves which characterize flagellum movement in trypanosomes, it is suggested that contraction may be co-ordinated by information from the kinetoplast.

The observations qualitatively support the assumption made by Bradfield[1] that conducting and contractile elements are present in a flagellum. No evidence is provided on the mechanism of transmission of information along a flagellum, nor is it yet possible to localize, within the fibre bundle, these functional elements of conduction or contraction.

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RADIOBIOLOGY

Fatty Acid and Glucose Metabolism in the Perfused Heart

Previous work on intact animals in which arteriovenous differences across the myocardium were measured[1,2] have suggested that fatty acids are an important metabolite for the heart. No work has been reported on the isolated heart with radioisotopes which allow a separation of the uptake and fate of a particular substrate. This communication gives an account of the uptake and certain metabolic fates of fatty acid and glucose in the isolated, perfused heart. The results indicate the readiness with which the myocardium utilizes both glucose and fatty acids, and suggest that fatty acids are preferentially oxidized. The latter observation provides a possible explanation for the puzzling increase in cardiac glycogen known to occur with fasting or with untreated diabetes.

The isolated perfused rat heart described by Bleehen and Fisher[3] was used. This was modified as indicated by Morgan and Park[4] to permit the use of a low circulating volume. To measure formation of carbon dioxide labelled with carbon-14 from labelled substrates, a closed system was developed which included 15 ml. of Krebs–Henseleit bicarbonate solution and about 100 ml. of a gas mixture (95 per cent oxygen : 5 per cent carbon dioxide). Glucose (5 m*M*) and free fatty acid (0·4 m*M*) concentrations were within the physiological range for the rat. A 0·5 per cent solution of dialysed human serum albumin was used in experiments with free fatty acids. Male albino rats weighing 200–240 gm. were killed by decapitation after an overnight fast. No anticoagulant was used. Hearts were perfused with a substrate-free Krebs–Henseleit solution for 5 min., during which time the coronary flow, force, and rate of contraction became stable. Perfusion for 30 min. in the closed system followed. Then the hearts were weighed and digested in 30 per cent potassium hydroxide. Non-saponified lipids were removed by a single petroleum ether extraction. Following acidification to *p*H 1, tissue fatty acids were recovered by three petroleum ether extractions. Analyses for glucose, glycogen and for incorporation of carbon-14 into carbon dioxide, glycogen and fatty acids were essentially the same as described by Winegrad and Renold[5] for adipose tissue. Lactate was measured enzymatically[6] and free fatty acids by Gordon's method[7].

A summary of results is shown in Table 1. It will be seen that free fatty acids (0·4 m*M*), when present as the only substrate, are readily removed by the heart. When the fatty acids were labelled with palmitate-1-^{14}C nearly half the uptake was found in carbon dioxide. Palmitate label was also recovered to a significant extent in 'tissue fatty acids'; whether

Table 1. UPTAKE AND FATE OF GLUCOSE-U-^{14}C (5 mM) AND PALMITIC ACID-1-^{14}C (0·4 mM) IN PERFUSED RAT HEART

Substrate	No. of hearts	Uptake Glucose	Uptake FFA	Incorporation of label into: CO$_2$		Glycogen		TFA		Lactate	Residual glycogen	Label accounted for
		μM	μM	μM	Per cent	μM	Per cent	μM	Per cent	μM	μM	Per cent
FFA*	8	—	4·14 ±0·3†	1·75 ±0·3	42 ±6	—	—	0·82 ±0·07	20 ±2	—	8·8 ±1·7	62
FFA* + glucose	6	10·0 ±4·7	3·96 ±0·1	2·12 ±0·1	54 ±2	0·006 —	0·07 —	1·00 ±0·09	25 ±2	—	11·8 ±0·7	79
Glucose* + FFA	10	23·7 ±1·8	4·88 ±0·2	0·52 ±0·08	2·2 ±0·1	0·73 ±0·28	3·1 ±1·0	0·005 (2)	0·005 (2)	36 ±6	11·9 ±0·8	54‡
Glucose*	12	29·8 ±1·2	—	5·1 ±0·8	17 ±2	0·44 ±0·05	1·45 ±0·2	0·01 —	0·02 —	43 ±8	9·6 ±0·9	62‡

All perfusions were for 30 min. Uptake, lactate production and label in CO$_2$, glycogen and tissue fatty acids (TFA) are expressed as μM/gm. wet weight/30 min.; residual glycogen as μM-glucose/gm. wet weight. Per cent = fraction of uptake.

FFA = free fatty acid (= unesterified = non-esterified fatty acid).

* ^{14}C labelled substrate.

† S.E. of mean.

‡ Sum of CO$_2$, glycogen and lactate (corrected for glycolysis during perfusion: in separate hearts the glycogen content after 5 min. perfusion with Krebs–Henseleit solution was 18·3 ± 1·2μM glucose/gm. wet weight) divided by uptake of glucose.

these represent fatty acids present in the tissue as free fatty acids or incorporation into triglyceride or other complex lipids has not been determined. Addition of unlabelled glucose (5 mM) to the perfusate had no effect on the uptake of free fatty acids; furthermore, the fraction of label from palmitate-1-^{14}C appearing in carbon dioxide and in tissue fatty acid was unchanged.

With glucose-u-^{14}C (5 mM) as the only substrate, 17 per cent of the uptake appeared as $^{14}CO_2$. There was definite incorporation of label into glycogen; essentially none was recovered as tissue fatty acid. Lactate production could account for 56 per cent of the glucose uptake and glycogen breakdown (Table 1). Addition of unlabelled free fatty acids (0·4 mM) strikingly reduced glucose oxidation to carbon dioxide. Under these conditions the uptake of glucose was slightly less, more residual glycogen was found after perfusion and more glucose carbon label was recovered in glycogen. No change in lactate production was observed.

The marked inhibition that free fatty acids exert on glucose oxidation to carbon dioxide suggests that fatty acids are preferentially oxidized when both substrates are present in the stated concentrations. It is unlikely that the reduced glucose oxidation to carbon dioxide produced by the addition of free fatty acids represents simply dilution of labelled carbon dioxide precursors by unlabelled substrate, since the addition of unlabelled glucose could not similarly alter the oxidation of palmitate-1-^{14}C. Furthermore, during free fatty acid inhibition of glucose oxidation, more of the glucose-u-^{14}C is incorporated into glycogen and the total myocardial glycogen is slightly increased. This suggests that the simultaneous presence of free fatty acids had diverted more glucose to glycogen synthesis with a net 'sparing' of cardiac glycogen.

Since fasting and untreated diabetes mellitus results in the mobilization of free fatty acids from adipose tissue these findings may explain the paradoxical observations that cardiac glycogen increases with fasting[7] and with untreated diabetes[8] in contrast to the depletion of glycogen in liver and diaphragm[9].

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HÆMATOLOGY

Site of Binding of Chromium-51 to Hæmoglobin

Gray and Sterling[1] have shown that when red blood cells are incubated with sodium chromate labelled with chromium-51, the latter enters the red cell and becomes firmly attached to intra-erythrocytic hæmoglobin. This tagging became the basis for *in vivo* studies of red cell volume and estimations of red cell life-span[2,3]. Despite its wide application in clinical medicine, little is known of the actual hæmoglobin-chromium-51 binding mechanism or site.

The molecule of normal adult hæmoglobin (A) has been shown to contain two pairs of distinguishable polypeptide chains (α and β) and can be schematically designated as $\alpha_2\beta_2$ (ref. 4). Most of the important abnormal hæmoglobins are altered only in the β chains. For example, hæmoglobin C can be represented as $\alpha_2\beta_2{}^C$ (ref. 5). Hæmoglobin H, an electrophoretically 'fast' variant, however, contains no α-chains and has been designated as β_4 (ref. 6). In acid solutions the hæmoglobin molecule undergoes asymmetric dissociation into free α_2- and β_2-units which

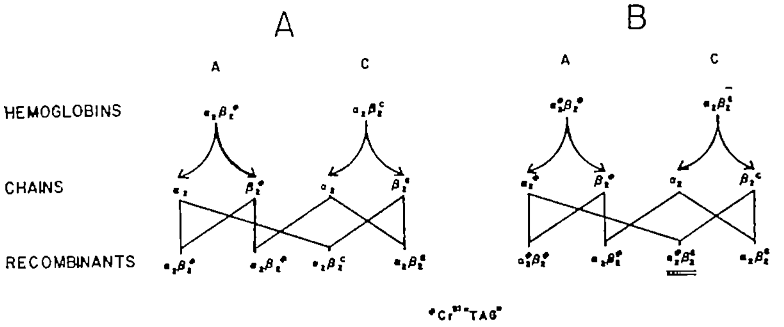

Fig. 1

recombine randomly when the solution is returned to neutral *p*H [7]. These facts have been utilized in this work in an attempt to elucidate possible sites of binding of chromium-51 to hæmoglobin.

Red cells from a patient with hæmoglobin *H* disease were tagged with iron-59 and chromium-51 as part of erythrokinetic studies. Hæmoglobin solutions were made by hæmolysis with toluene and cleared by ultracentrifugation. Oxyhæmoglobins *H* and *A* were separated by starch-block electrophoresis in phosphate buffer *p*H 7·0 in a 3-hr. run at 50 m.amp. and 400 V. The separated components were then analysed for hæmoglobin content and for chromium-51 and iron-59 activity by γ-ray spectrometry in a sodium iodide well-type scintillation counter. Results are presented in Table 1.

Table 1

	Per cent of total hæmoglobin	c.c./min. ^{59}Fe	Per cent of total ^{59}Fe counts	c.c./min. ^{51}Cr	Per cent of total ^{51}Cr counts
Hæmoglobin *H*	18	16	20 5	1,107	33 7
Hæmoglobin *A*	82	62	79 5	2,178	66 3

This indicates that the chromium-51 activity of hæmoglobin *H* (β_4) is approximately twice that of equivalent amounts of hæmoglobin *A* ($\alpha_2\beta_2$).

One explanation for the differences in chromium-51 activities of hæmoglobin *H* and *A* would be that chromium-51 tags primarily to β-chains. To test this hypothesis, recombination experiments with chromium-51-labelled hæmoglobin *A* and non-radioactive hæmoglobin *C* were performed.

If chromium-51 binds only to β chains no radioactive hæmoglobin *C* would be seen after recombination, since no 'hybrids' would be formed (Fig. 1*A*). On the other hand, if chromium-51 becomes attached non-specifically to both α- and β-chains, radioactive hæmoglobin *C* would result, due to 'hybridization' (Fig. 1*B*).

Red cells from a normal person were labelled with chromium-51 and hæmolysed immediately. Hæmoglobin *C* was obtained from the hæmolysate of a patient with homozygous hæmoglobin *C* disease. Equal amounts of these solutions were mixed and then divided into control and reaction portions. Hæmoglobin concentration of these was approximately 5·0 gm. per cent. The reaction portion was dialysed for 12 hr. against acetate buffer *p*H 4·7 and then returned to basic *p*H by successive dialysis against distilled water and barbital buffer *p*H 8·6. A precipitate was removed by high-speed centrifugation. Both control and reaction mixtures were converted to cyanmethæmoglobin and separated by starch-block electrophoresis in barbital buffer at *p*H 8·6 in a 16-hr. run at 48 m.amp. and 350 V. The fractions were then eluted and analysed for hæmoglobin concentration and chromium-51 activity. Results are listed in Table 2.

Table 2

	Per cent hæmoglobin		c.c./min. ^{51}Cr		Per cent ^{51}Cr activity	
	A	*C*	*A*	*C*	*A*	*C*
Control mixture	39 3	60·7	955	38	96·2	3 8
Reaction mixture	42 5	57 5	4,069	226	94·6	5 6

Similar results were obtained in experiments using hæmoglobin *S* and agar electrophoresis. The reason for the chromium-51 activity in the hæmoglobin *C* fraction is uncertain. Approximately 3 per cent can be accounted for by hæmoglobin A_2 present in the *A* hæmolysate and tagged along with hæmoglobin *A* (unpublished observations).

Using two different experimental approaches, it has been shown that chromium-51 is attached to hæmoglobin largely on the β-chains. Since the chromium-51 tag remains with the β-chain during dissociation and recombination, this may provide a useful tool for localizing altered polypeptide chains of abnormal hæmoglobins.

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IMMUNOLOGY

Characteristics of γ-Globulin, lacking Antibody Properties, in New-born Pigs

IN solving the problem whether γ-globulin lacking antibody properties exists and whether γ-globulin can be formed without an antigenic stimulus, our attention was focused on γ-globulin in piglets which had not been fed with colostrum[1]. The serum of piglets bred by a special technique without colostrum[2] is very suitable material for these experiments, because the placenta of these animals does not permit the penetration of γ-globulin[3], and the new-born animals lack this protein completely[4-6]. In our previous experiments[1], however, the presence of a detectable amount of γ-globulin has been demonstrated after 100 times concentration of the γ-globulin fraction of the serum taken from the piglets bred under sterile conditions without colostrum for the first 14 days after birth; at that time piglets are not yet able to produce antibodies, not even against an artificially injected antigen (*Brucella suis*) nor against antigens naturally present (*E. coli* strains). This γ-globulin cannot combine with any of the tested antigens and it could therefore be described as 'normal' γ-globulin. By injecting methionine labelled with sulphur-35 to piglets bred without colostrum it was found that this small amount of γ-globulin was not transmitted passively from the mother by the intra-uterine route, but that the γ-globulin of the young animals was synthesized *de novo* during the period when the piglets were not yet producing antibodies.

In the present communication the results of physicochemical and immunochemical investigations of γ-globulin of piglets which were bled shortly after birth without the intake of colostrum are presented. The γ-globulin was isolated from the serum by twice-repeated preparative electrophoresis on a starch block in veronal buffer, *p*H 8·6, $\Gamma/2 = 0{\cdot}05$. The yield of the product which we called γ_N (γ-globulin neonatorum) was 50–80 μgm. from 1 ml. of the serum. Paper and agar electrophoresis revealed a microheterogeneity typical of γ-globulins. The admixture of β-globulin was not detectable by paper and agar electrophoresis; its presence was demonstrated only using immunoelectrophoresis. Ultracentrifugation analysis indicates that the preparation γ_N contains a large component, apparently of a microheterogeneous nature, with a mean corrected sedimentation coefficient $S_{20,w} = 3{\cdot}1\ S$, and a small component with a sedimentation coefficient $S_{20,w} = 6{\cdot}1\ S$, contrary to the γ-globulin of adult pigs, which contains only components of 6·7 *S* and 18 *S*. The preparation was subjected to chromatography on *DEAE* cellulose in phosphate buffer, *p*H 8·0, with an ionic strength gradient according to Fahey[9]. The distribution of the proteins in the fractions is shown in Fig. 1. In order to carry out the immunochemical analysis of the separated fractions, two rabbit antisera were prepared. The first one was obtained by immunization of rabbits with electrophoretically prepared γ_2-globulin of adult pigs and was called anti-*P*γ*GA*. The second one (anti-*PGN*) was obtained by immunization of rabbits with a fraction isolated by starch block electrophoresis from the γ-globulin area of new-born pig serum and containing γ- and β-globulins. Each chromatographic fraction was subjected to the agar precipitation test in Preer's modification. The precipitate with anti-*P*γ*GA* serum appeared in fractions 13–30. This was a guide for making a sub-fraction $\gamma_{N'}$ which contained non-precipitating protein from the large frontal peak (about 50 per cent of protein γ_N) and $\gamma_{N''}$ containing protein precipitating with anti-*P*γ*GA* (about 20 per cent protein γ_N). The sedimentation coefficient $S_{20,w}$ was 2·7 *S* for $\gamma_{N'}$ and 5·1 for $\gamma_{N''}$. If $\gamma_{N'}$ is precipitated in immunoelectrophoresis with antiserum against piglet β- and γ-globulin (anti-*PGN*) at least four different components appear. Anti-*P*γ*GA*, similarly as in Preer's modification, does not form any precipitate with $\gamma_{N'}$ in immunoelectrophoresis. That means that no antigenic determinant of $\gamma_{N'}$ is identical with γ-globulin of adult animals. $\gamma_{N''}$ precipitates in a single line with either of the antisera used. Anti-*PGN* precipitates also with adult γ_2-globulin, showing a double precipitation line. This is further evidence that serum of new-born pigs contains proteins which possess at least some determinants of γ-globulin.

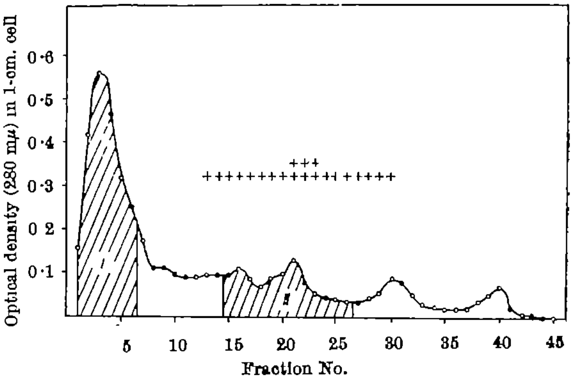

Fig. 1. Chromatography of γ_N on *DEAE* cellulose. Weight of protein, 25 mgm. Gradient from 0 02 *M* sodium phosphate, *p*H 8·0, to 0·3 *M* sodium phosphate, *p*H 8·0. Fractions in which precipitate with anti-*P*γ*GA* serum was formed are expressed by crosses

To elucidate completely the antigenic nature of γ_N and its relationship to γ_2 of adult animals a quantitative precipitation of anti-*P*γ*GA* serum with antigens $\gamma_{N''}$ and γ_2 was carried out. The results of the precipitation in Fig. 2 show the sharp peak, contrary to the high and broad zone of maximal precipitation with $\gamma_{N''}$. Although we cannot rule out that the preparation of $\gamma_{N''}$ contains protein which does not participate in the precipitation with anti-*P*γ*GA* (for example, an admixture of β-globulin) the precipitation curves indicate quite clearly that $\gamma_{N''}$ is not fully identical with γ_2. If this were the case, both precipitation curves would fuse or have the same shape and be shifted on the antigen axis in the co-ordinate system used in Fig. 2. Since $\gamma_{N'}$, which contains the components with the smallest sedimentation coefficients from the whole mixture, does not precipitate at all with anti-*P*γ*GA*, it was investigated whether its possible antigenic relationship with γ-globulin will manifest itself by inhibition in the precipitation of anti-*P*γ*GA* with γ_2 in the presence of excess of $\gamma_{N'}$. In this test, $\gamma_{N'}$ behaved as a quite indifferent protein, and there is thus no evidence of its immuno-chemical relationship with γ-globulin.

Immunochemical data, together with the sedimentation and electrophoretic characteristic, thus indicate

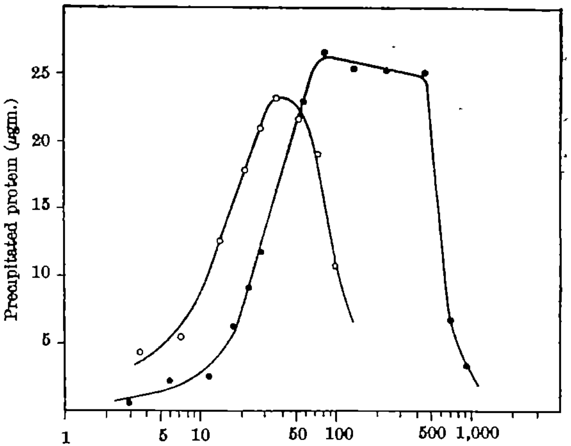

Fig. 2. Precipitation curves of anti-*P*γ*GA* serum versus adult γ_2-globulin (open circles) and $\gamma_{N''}$ protein (solid circles)

that the serum protein of new-born pigs which is electrophoretically identical with γ-globulin contains components in close antigenic relation with γ-globulin of adult animals but immunochemically not identical. These components differ from γ-globulins of adult animals also by their molecular weight as shown by determination of a significantly lower sedimentation coefficient.

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Effect of some Metabolic Inhibitors on Antibody Formation

Recently the use of metabolic inhibitors, particularly analogues of nucleic acid components, has been proving an effective tool for investigating the processes involved in antibody formation[1-8]. The direct determination of the metabolic changes attendant on these processes is practically impossible at the present stage. After the first immunizing dose, only a very small proportion (about 1 per cent) of the lymphoid cells isolated from the spleen or the lymph nodes is active in antibody formation. The detection of any biochemical changes in these cells is made exceedingly difficult not only by the large 'background' of inactive cells (99 per cent) but also by the absence of serologically demonstrable antibody from the active cells in the initial (inductive) phase. Earlier I showed that the nucleic acid antimetabolite 6-mercaptopurine acts only during this inductive phase, but does not affect antibody production if administered during the productive phase[9]. If, then, antibody formation is inhibited by means of suitable agents without affecting protein synthesis in general, we may with some degree of plausibility draw conclusions about the processes taking place in the inductive phase.

The work to be described here, in which the effect of a variety of inhibitors was examined, was intended chiefly to show whether these inhibitors act directly by interference with intracellular metabolism, or rather indirectly through the inhibition of mitotic cell division resulting from their primary action. If it were possible to show that the inhibition of antibody formation is directly connected with the reduction of mitotic activity then this would constitute evidence for the validity of the clonal selection theory at the cellular level. A further objective of this work was, of course, the hope of finding compounds more effective in suppressing immunological response and less toxic than 6-mercaptopurine.

Table 1. Action of Metabolic Inhibitors on Antibody Formation

Type of inhibitors	Compound	Dose (mgm./100 gm. body-weight)	Inhibition of antibody formation
Pyrimidine antagonists	5-Fluorouracil	2·0*	0
		1·0	0
		0·5	0
	5-Bromouracil	5·0	0
	6-Azauracil	2·0	0
		1·0	0
		0·5	0
	6-Azauridine	500·0*	+
		50·0	±
		5·0	0
	6-Azauridine-monophosphate	4·0	0
	2-Thiouracil	5·0	0
		10·0	0
	4-Methyl-2-thiouracil	1·0	0
		5·0	0
	6-Azathymine	0·5	±
	2-Thio-6-azathymine	2·0	0
	2-Thiothymine	2·0	0
Purine antagonists	6-Mercaptopurine	0·5*	+
		0·3	+
	8-Azaguanine	1·0	±
		0·5	0
	6-Thioguanine	0·1*	+
		0·05	+
Folic acid antagonist	Aminopterine	0·3	+
		0·15	+
		0·05	±
Inhibitors of mitotic division	Colchicine	0·3*	0
		0·2	0
	Demecolcine	0·1	0
	Actinomycin *C*	0·003*	0
Amino-acid antagonist	DL-Ethionine	5·0	0
Polyfunctional alkylating agents	*TS*-160 (trichlorethylamine)	0·1	0
	Myleran	2·0*	+
		0·5	0
	Leukeran	0·5	0

0, No significant inhibition of antibody formation; ±, inhibition significant in some animals; +, total inhibition in all cases.

*, Dose causing distinct toxic effects for the recipients of the spleen cells—new-born rabbits.

Inhibitory activity was determined by the same method as had been used in previous work with 6-mercaptopurine[9]. The cells isolated from the spleen of a normal non-immunized donor were mixed *in vitro* with *Brucella suis* antigen and injected intraperitoneally in a dose of 50 × 10^6 cells into three- to five-day-old rabbits. The antibodies produced by the transferred cells reach their highest titre 5–7 days after transfer, that is, at that stage in the development of the recipient when the latter is still incapable of responding to the antigen injected by antibody formation[10]. The evaluation of antibody response and its inhibition was confined to that stage (that is, the tenth to twelfth day of life of the recipients). The inhibitors were injected daily, for five days after transfer of the cells, at various dose-levels as indicated in Table 1 (only doses which were not yet fully toxic for the animal are recorded). In all, 400 neonatal rabbits were used in these experiments. The use of neonatal animals, which are more sensitive to the action of inhibitors[11], made it possible to distinguish more precisely between the general cytostatic effect of the inhibitors and their selective action on antibody formation.

The inhibitors used included some analogues of the purine and pyrimidine bases, of folic acid, and of methionine, as well as some known cytostatics and polyfunctional alkylating agents. Table 1 summarizes the effects of some of these compounds. Inhibitory activity is found predominantly among the purine derivatives, whereas the pyrimidine derivatives do not inhibit antibody production. 5-Fluorouracil had no effect on antibody production at doses which are known to be carcinostatic[12] and which were toxic for the neonatal rabbits. The only active pyrimidine antagonist was 6-azauridine (kindly supplied by Dr. J. Škoda, of the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Science; cf. ref. 13), but only in large, toxic doses (500 mgm./100 gm. body-weight). Since, of the purine analogues, the sulphur-substituted compounds were the most active, I also investigated a number of thio-compounds based on the pyrimidine skeleton (the compounds were kindly supplied by Dr. J. Gut, of the same Institute; cf. ref. 14); but the introduction of sulphur into the molecules did not lead to any enhancement of the activity.

The action of compounds known to inhibit cell division gave further information relevant to the question whether the formation of a cell clone is a pre-requisite for the induction of an antibody reaction. Injection of colchicine and actinomycin *C* in the highest possible doses was found not to affect the antibody response. These results accord with those obtained with 5-fluorouracil, which was also inactive in inhibiting antibody formation in doses which were generally cytotoxic.

Since other experiments have also indicated that the essence of the inductive phase does not lie in the multiplication of a predetermined type of cells under exposure to the antigen[15], it may be concluded that the effect of the active purine analogues on antibody formation involves direct interference with the adaptive metabolic processes which take place during the inductive phase.

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PATHOLOGY

Tissue Responses to Filter Membranes of Various Porosities

In the course of work on experimental silicosis in which diffusion chambers were used, it was discovered that the reaction of the tissues to the chamber varied greatly with the pore-size of the membrane of which its walls were made. The chambers were of the type used in earlier experiments[1] and the membrane was 'Millipore' type. The porosities tested were 0·65μ, 0·8μ, 1·2μ, 3μ and 5μ.

When placed within the rat's peritoneal cavity, each chamber is covered with mast cells and polymorphs within an hour. The mast cells rapidly disrupt and the granules enter the pores of the membrane; wherever the pores are full of 'cement', however, these cells remain intact. Within the next few hours large numbers of polymorphs enter the pores of the membrane, but comparatively few succeed in reaching the lumen of the chamber; most of them undergo rapid dissolution in the pores. Simultaneously, an inflammatory reaction develops in the adherent tissue which gradually increases until it is most intense after about three days. Thereafter, a fibrous reaction gradually develops so that, after 14 days, a dense fibrous capsule encloses the chamber. Half-chambers excite the same response as whole chambers (Figs. 1 and 2). The inflammatory reaction is most acute with membranes of 1·2μ, 3μ or 5μ porosity. With 0·8μ-pore membrane, however, the reaction is less acute: fewer polymorphs enter the pores and die there; no intact polymorphs reach the lumen of the chamber; the inflammatory reaction in the adherent tissue is less, and the fibrous capsule that forms is thinner. With 0·65μ-pore membrane, the majority of chambers do not even adhere to the omentum or mesentery: only a small number of polymorphs manage to enter the pores and, consequently, only a small amount of nuclear debris collects in the pores; the fibrous capsule is quite delicate.

Soaking the filter membrane in various solvents fails to make it less inflammatory. The only way to make it non-irritant to the tissues is to block the pores, either with 'cement' or by softening the membrane with acetone. In any event, the membrane is not toxic to cultures of *Strept. pyogenes*. There is no evidence therefore for the presence of a toxic contaminant.

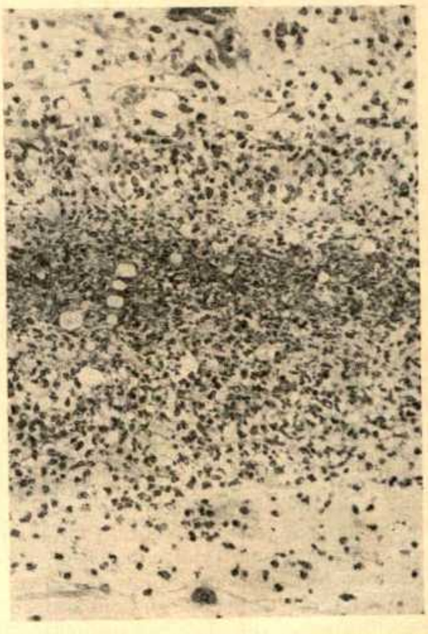

Fig. 1. Half-chamber made with 1·2μ-pore membrane after 2 days intraperitoneally. The membrane runs across the centre but is buried in inflammatory exudate. It is represented by the dark-staining band, which consists of the debris of polymorph nuclei within its pores. Inflammatory exudate covers both surfaces. (Hæmatoxylin and eosin. × 100)

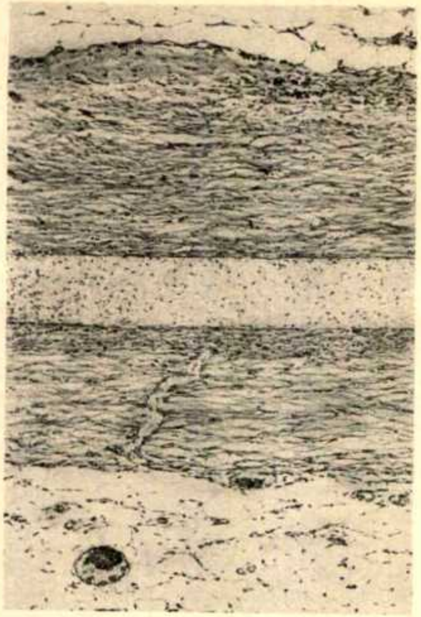

Fig. 2. Half-chamber made with 1·2μ-pore membrane after 14 days intraperitoneally. The membrane (centre) is now enclosed by a dense mass of fibrous tissue. Its pores contain only a few polymorphs. (Hæmatoxylin and eosin. × 65)

This acute inflammatory and fibrotic reaction was unexpected, because earlier experiments[1,2] showed that chambers made with 0·1μ-pore (or even 0·45μ-pore) 'Millipore' membrane did not adhere to tissues or excite an inflammatory reaction ; and all 'Millipore' filters have the same chemical composition and the same weight of cellulose ester for a given size of filter, the only variable being the pore size. Since it seems that the intensity of the inflammatory reaction correlates with the ease with which polymorphs can enter the pores and die therein, the membranes may produce their effect by acting as a polymorph 'trap'. Clearly, if the dying polymorphs released an 'inflammatory' agent in the form of a vascular permeability-increasing factor, an inflammatory reaction would build up rapidly. This would be terminated only when fibrous tissue had sealed off the pores. On the other hand, there is the possibility that the migration and death of the polymorphs is secondary and merely one aspect of an acute inflammatory response initiated in some other way by the membrane. Permeability factors are becoming embarrassingly numerous and can be readily activated ; for example, contact of serum with diethylaminoethyl cellulose during chromatography is sufficient to release one[3]. But, if the membranes are showing surface activity and releasing a permeability factor from the tissue fluids, it is difficult to explain why only the coarser-pore membranes do so, since, presumably, they expose a much smaller surface area to the tissue fluids than do membranes with finer pores (less than 0·45μ). Whatever the mechanism, it is interesting to find a chemically pure and 'inert' material capable of causing an acute inflammatory response and subsequent fibrosis because of a slight alteration in its physical form.

The significance of the mast cell response is difficult to assess. It was not caused by trauma, because these cells were intact over the 'cement-filled' areas. Since they do not react to smaller-pore (0·45μ or less) membranes, it is tempting to link them with the initiation of the inflammatory reaction ; and, of course, they are well equipped to do so, since they contain histamine as well as serotonin and heparin. However, there is little other evidence to link mast cells with acute inflammation.

If disintegrating leucocytes could release a permeability factor, it would have a bearing on the problem primarily under consideration in these experiments, namely, how silica induces fibrosis. Little is known about the chemical mediators in chronic inflammation and fibrous repair, but it seems reasonable to assume that both processes are essentially manifestations of the sustained release in the tissues of a permeability factor. Such a factor would maintain a steady flow of fibroblasts and leucocytes from patent capillaries until sufficient fibrous tissue has formed to wall off the irritant. On this basis silica dust might be unique, not in its influence on fibroblasts, as is often assumed, but only in its ability to persist in the tissues and disintegrate the leucocytes which ingest it.

A more detailed account of this work will be published[4].

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BIOLOGY

Proposed Revisions of 'Transplantese'

The jargon employed by students of tissue transplantation has been named 'transplantese' by one of us[1].

Within a given species we wish to distinguish grafts exchanged between genetically identical individuals from those between genetically diverse individuals. Snell[2] suggested the word 'isograft' for the former and 'homoio-transplant' for the latter. Many people have remarked on the confusion arising from the use of isograft, which connotes genetic or antigenic uniformity, and 'isoantigen', which connotes the reverse. The latter is by far the older term and widely used by immunologists. As has been pointed out previously, transplantation immunity is but one facet of immunology, and a minority should try to conform to the terminology employed by the majority. There is also a more serious consideration which has been overlooked. The word 'isos' means 'equal', and 'homos' and 'homoios' mean 'the same'. It is therefore not logical to make the prefix 'iso-' denote a greater degree of similarity than 'homo-' or 'homoio-'.

Grafts between species are of less general interest. They have been called xenografts or heterografts.

Three adjectives related to the above nouns are in common use : 'isologous' (=genetically identical), 'homologous' (=of the same species, but not genetically identical) and heterologous (=of different species). Recently 'autologous' has disfigured the literature. This is not the place to discuss the origins and meaning of the termination '-logous', but it is not suitable for defining genetic relationships. 'Homologous' should always be used to denote some type of agreement or correspondence. There is no possible justification for using the word to mean 'genetically different' since it is a flagrant contradiction of its proper use. 'Isologous' has no popular meaning; but, for the reasons stated above, it should not be made to denote a closer similarity than 'homologous'. 'Heterologous' is the correct antithesis of 'homologous', but it has no necessary reference to inter-specific differences. Indeed immunologists often use 'homologous' and 'heterologous' without any genetic connotation. The 'homologous' antigen is generally that used for immunization, and another antigen, even if derived from the same individual as the former, could be described as heterologous. The terms are often so used in discussions concerned with synthetic antigens. 'Autologous' is both meaningless and unnecessary.

One of us[1] suggested 'isogenic', 'allogenic' and 'heterospecific' in place of 'isologous', 'homologous' and 'heterologous', and we should like to reconsider these terms here.

So far as we can discover 'isogenic' was originally used to denote a population that was homozygous for all genes. The meaning seems to have been tacitly extended to cover other types of genetically uniform populations such as monozygotic twins and the F_1 between inbred strains. This being so there can be no possible objection to the use proposed above. However, we feel there is a need for a synonym that avoids the prefix 'iso-'. This is particularly needed with tumours. It is notorious that in the past so-called tumour-specific antigens have turned out to be isoantigens. There are now several examples of intra-strain immunity to tumours and it is most unfortunate that authors such as Hirsch[3] have used 'isoimmunity' in this context. We suggest the introduction of 'syngeneic' as a synonym for 'isogenic'. If one does not wish to refer to 'intra-strain immunity' one could then use 'syngeneic immunity'.

No objections have been raised against 'allogenic' as written, but in conversation it can be confused with 'allergenic'. We therefore propose 'allogeneic'; this should be pronounced 'allogeneeic', but even if pronounced 'allojeenic' it should not sound like 'allergenic'.

'Hetero-specific' has been used before and cannot be said to be illogical; but it is a Greco-Latin hybrid and we feel that 'xenogeneic' goes well with the other two terms we have suggested and is perhaps preferable.

We see no reason for any modification of 'autograft'. The adjective 'autoplastic' has been in use many years, and it does not seem justifiable to abandon it. A tumour growing in its original host has long been spoken of as 'autochthonous'.

'Homograft' and its commonly used synonyms are too firmly established and too useful to abandon. It must be realized that the term should embrace all types of intra-specific grafts and cannot be reserved for grafts between genetically diverse individuals. We cannot say that 'isograft' has proved a very fortunate term. Snell[2] originally intended it as an abbreviation of 'isogenic graft' although very few people seem aware of the fact. The expanded form is certainly preferable to that generally used. 'Isogenic homograft' is more precise. For reasons given above we feel there is much to be said for the employment of 'syngeneic homograft or graft'. 'Allogeneic graft' could, and probably will, be abbreviated to 'allograft'; but recent experience does not encourage premature abbreviation.

Table 1. PROPOSED REVISIONS OF 'TRANSPLANTESE'

Nouns			Adjectives		
Old terms	Proposed new terms	Alternative new terms	Old terms	Proposed new terms	Alternative new terms
Autograft	Autograft	—	Autologous	Autoplastic autochthonous (acc. to context)	
Isograft	Syngeneic homograft or graft	Isogenic homograft or graft	Isologous	Syngeneic	Isogenic
Homograft (general sense)	Homograft	—	Homologous	Allogeneic	—
Homograft (connoting genetic diversity)	Allogeneic homograft or graft	—			
Heterograft	Xenograft	Heterospecific graft Interspecific graft Heterograft	Heterologous	Xenogeneic	Heterospecific Heterologous

'Heterograft' is a relatively inoffensive term; but the meanings of 'heteros' and 'allos' are similar and do not provide a suitable contrast. We feel that the old term 'xenograft' is more explicit. The proposed alterations are shown in Table 1.

We should like to see international agreement on the terms to be used in this interesting field of study; but we cannot see any justification for continuing to antithesize sameness and equality in the perverse way that has commonly been done.

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Electrophoretic Patterns of Lymph Node Proteins in Hybrid F_1 Mice irradiated and treated with Myeloid and Lymphoid Cells from a Parental Strain

IT is generally admitted that graft antibodies do not circulate in the plasma and that graft immunity is carried on by cells from the lymphoid tissue and more precisely by those 'immunologically competent cells' of which the most striking features are their plasma cell-like morphological aspect and their high content of ribonucleic acid[1].

The purpose of this work was to find out whether graft immunization in lymph nodes will result in a modification of the electrophoretic pattern of the lymph node proteins as investigated by means of starch-gel electrophoresis[2]. It is implied that eventual modifications in patterns could correspond to the graft antibody located inside the immunized cells.

(*a*) In a first group of experiments, we have studied the lymph nodes of the host in connexion with a graft of lung tissue. The nodes were removed 5–7 days after insertion of the graft. Genetically the systems used herein were as follows: Host: *C57Bl6*; donors: *DBA2*. Hosts: *DBA2*; donors *C57Bl6*. Hosts: *C57Bl6*; donors $(C57Bl6.\ DBA2)F_1$.

In all these cases the lymph node content in hyperbasophilic cells was moderately increased, up to 4 and 6 per cent. The electrophoretic patterns of the lymph node proteins were apparently similar to those of lymph nodes from non-grafted mice, *C57Bl6* or *DBA2* or F_1 taken as controls and also to those of mice grafted with isogenic lung grafts. In the latter the number of cells with hyperbasophilic cytoplasma was normal.

(*b*) In a second group of experiments, we have studied the lymph node proteins of $(C57Bl6.DBA2)F_1$ receiving a whole-body irradiation of 500 rads (200 kV., 50 cm. distance, 12 m.amp., 0·5 aluminium, 0·5 copper) and injected 24 hr. later with 2×10^7 bone marrow cells and 2×10^7 lymph node cells from the parental strain *C57Bl6*.

In this group, we actually observed in the lymph nodes a definite reaction of immunologically competent grafted cells against the antigens of the host. The number of hyperbasophilic cytoplasm cells seven days after grafting was considerable, reaching a percentage of more than 40 (ref. 3). When extracts of these lymph nodes were submitted to electrophoresis through starch gel, an extra band (Fig. 1) appeared which does not exist in extracts of lymph nodes from untreated mice nor from mice injected with bone marrow and lymph nodes cells from isogenic donors: F_1 injected with F_1 cells, *C57Bl6* injected with *C57Bl6* cells.

Within the limits of our experiments, it can be asserted that this particular band shows only when: (*a*) there is a transplantation immunological reaction which in this particular case was a reaction of the grafted cells against the antigens of the host that the grafted cells did not possess; (*b*) when the number of hyperbasophilic cytoplasm cells is very high.

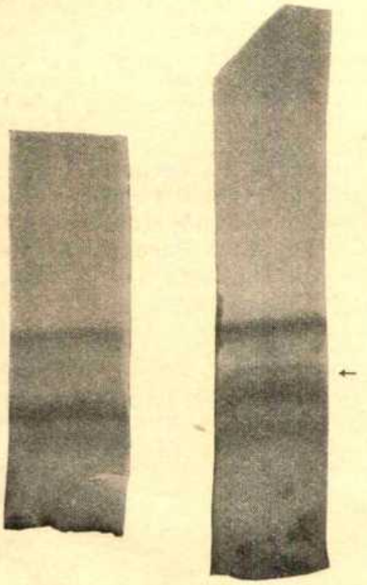

Fig. 1

Only the cathodic fraction of the gel is represented on the figure

It is not unreasonable to assume that the band shown in starch gel whenever an immunity reaction takes place in the lymph nodes together with the striking increase in number of hyperbasophilic cytoplasma cells might correspond indeed to the production of the antibody.

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Lysine Synthesis and Phylogeny of Lower Fungi: Some Chytrids versus *Hyphochytrium*

RECENT work, conducted with the aid of various diagnostic carbon-14 tracers, has suggested that lysine biosynthesis is a valuable character in the detection of certain evolutionary relationships[1–4]. All the eubacteria, pseudomonads, actinomycetes[1], green algæ[2], and higher (vascular) plants[3] examined appear to synthesize lysine in one general manner (presumably via α,ε-diaminopimelic acid, cf. ref. 5) and all the ascomycetous and basidiomycetous fungi[1,3] (and also *Euglena*[2]) in another (presumably via α-aminoadipic acid, cf. ref. 6). Among the lower fungi (phycomycetes), a striking dichotomous distribution of the two lysine paths was noted: the Mucorales and Blastocladiales studied, like the higher fungi, evidently use the path via α-aminoadipic acid, and the Saprolegniales that via α,ε-diaminopimelic acid[4]. The present communication reports findings with some other phycomycetes, including three chytrids and a representative of the Hyphochytriales. The latter group of organisms, although closely paralleling the chytrids in body structure, have uncertain phylogenetic affinities.

The formation of lysine in the phycomycetes selected was investigated with the aid of radioactive DL-aspartate, DL-alanine, and acetate. Table 1

Table 1. INCORPORATION OF TRACERS INTO PROTEIN AMINO-ACIDS OF *Rhizophlyctis rosea* AND *Hyphochytrium catenoides* (AS SPECIFIC ACTIVITY RELATIVE TO THE RESPECTIVE PROTEIN ASPARTIC ACID VALUES)

Organism	Tracer	Aspartic acid	Threonine	Lysine
R. rosea	[4-^{14}C] Aspartate	100	104	< 5
R. rosea	[1-^{14}C] Alanine	100	95	< 5
R. rosea	[2-^{14}C] Acetate	100	91	440
H. catenoides	[4-^{14}C] Aspartate	100	94	107
H. catenoides	[1-^{14}C] Alanine	100	91	134
H. catenoides	[2-^{14}C] Acetate	100	90	90

The organisms were cultivated in 20 ml. of glucose-salts medium in 250-ml. flasks at 28° C., *R. rosea*, without shaking, in the medium of Stanier (ref. 7) and *H. catenoides*, with shaking, in that of Fuller (personal communication). Approximately 2–5 mgm. (20–50 μc.) of tracer were supplied per flask. For the determination of relative molar amounts of the three protein amino-acids, experiments with uniformly labelled glucose were performed. The cultures obtained were subjected to the collecting, extraction, hydrolysis, paper chromatography, and counting procedures previously described, and specific radioactivities of the protein amino-acids were computed on a molar basis, as before (refs. 3 and 8).

shows the labelling pattern found for the chytrid, *Rhizophlyctis rosea*, strain *QM*, obtained through the courtesy of Dr. J. N. Couch, and for a strain of *Hyphochytrium catenoides*, isolated by Dr. Couch and kindly furnished by Dr. M. S. Fuller. In the case of the former organism, the 4-carbon of aspartate and the 1-carbon of alanine are not appreciably incorporated into protein lysine, whereas the 2-carbon of acetate labels lysine heavily (in comparison with the labelling of protein aspartic acid and threonine). Essentially the same behaviour was shown by two other chytrids (generously provided by Dr. Couch and Dr. Fuller, respectively): *Phlyctochytrium punctatum*, strain *NG*-3 (ref. 9) and an organism similar to strain *QM*. In the case of *H. catenoides*, the 4-carbon of aspartate and the 2-carbon of acetate make approximately equal relative contributions to protein lysine, aspartic acid, and threonine; the 1-carbon of alanine contributes somewhat more extensively to lysine than can be accounted for by the conversion of the alanine to aspartic acid. In experiments with DL-[3-^{14}C] aspartate, the chytrids as well as *H. catenoides* showed substantial incorporation of carbon-14 into lysine. With regard to these labelling patterns, which will be discussed in more detail elsewhere, the chytrids are like the Blastocladiales, Mucorales, and higher fungi (as well as *Euglena*) and presumably have the α-aminoadipic acid–lysine path, whereas *H. catenoides* resembles the Saprolegniales and green algæ (as well as bacteria and vascular plants) and appears to use the α,ε-diaminopimelic acid–lysine path. It is noteworthy that the path via α-aminoadipic acid has thus far been indicated exclusively in those phycomycetes producing posteriorly uniflagellate zoospores or aplanospores, and the path via α,ε-diaminopimelic acid in those producing biflagellate or anteriorly uniflagellate zoospores.

The present results strengthen the view that the mode of lysine synthesis is a useful character in the exploration of certain phylogenetic links. The utility of this character is thought to depend in part on the fact that each of the two pathways contains a number of enzymes that appear to be specifically concerned with the formation of lysine. The two sets of enzymes are thus presumed to represent a considerable period of evolution and are viewed as biochemical organelles of ancient origin.

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Growth of Embryonic Mammalian Cartilage after Freezing

SINCE the discovery that glycerol will protect cells and tissues against freezing damage[1], considerable progress has been made in developing techniques for the preservation of tissues at very low temperatures. Attempts to establish low-temperature banks of adult human cartilage have unfortunately, so far, proved unsuccessful[2]. Biggers[3], however, was able to show that embryonic chick tibiæ will grow and differentiate *in vitro*, after freezing to −79° C., in saline containing 15 per cent glycerol. This communication describes briefly some of the work concerned with freezing embryonic rat tibiæ to −79° C. A fuller account is being given elsewhere[4]. Rat fœtuses of a known age were obtained from the Medical Research Council strain of albino rat, bred in the Department. The occurrence of a vaginal copulation plug, or the presence of sperm in the vaginal smear, was taken as the indication of mating. On the seventeenth day *post coitum*, the conceptuses were excised, and the embryonic tibiæ were isolated under a dissecting microscope. The bones were cleaned of as much extraneous tissue as possible, with care to avoid injuring the perichondrium. The structure of these tibiæ is being described elsewhere[5].

The embryonic bones were cultivated *in vitro* using either the plasma clot technique[6] or the floating-lens paper method[7]. The cooling technique employed was that described by Smith[8], and in all cases the frozen bones were thawed rapidly.

A preliminary series of experiments investigated the growth of 17-day embryonic rat bones after they had been equilibrated for various lengths of time in saline or plasma containing 15 per cent glycerol and then either frozen or explanted without freezing. Both the frozen and the unfrozen bones were rapidly rinsed in Tyrode's solution to remove any residual glycerol before they were explanted. In all these experiments, the frozen bones showed no subsequent elongation *in vitro*. Of the unfrozen bones, those which had been exposed to a concentration of 15 per cent glycerol, for periods not exceeding 30 min., elongated in a normal manner; those unfrozen bones that had been exposed to higher concentrations of glycerol-plasma, or for extended equilibration periods, did not afterwards elongate.

It was then decided to use trypsin, in an attempt to discover whether chondrocytes which were partially freed from their matrix would survive freezing. Tibiæ and femora from 17-day-old embryonic rat tibiæ were chopped into two or three pieces and digested with trypsin, using the method of Moscona and Moscona[9]. Tryptic digestion was carried out until the matrix was viscous and jelly-like, but the cells were still embedded in the matrix. The trypsin-treated cartilage was then suspended in 15 per cent glycerol plasma, and equilibrated for 30 min. at room temperature. Two thirds of the fragments were then cooled slowly to −79° C. and kept at this temperature for 1 hr. The remaining unfrozen fragments were briefly rinsed in Tyrode's solution to remove any residual glycerol and explanted. The frozen fragments were thawed rapidly, rinsed in Tyrode's solution, and explanted. Each culture contained fragments from approximately two bones. The explants were cultivated for five days and were sub-cultured after three days *in vitro*. Cartilage which survived the experimental treatments appeared healthy and viable; the amorphous fragments rounded up into whitish opaque nodules, regaining a definite shape after approximately 12 hr. *in vitro*. The nodules increased in size during the cultivation period, and, in addition, there was a peripheral outgrowth of clearly visible fibroblasts, growing in a radial pattern.

A similar experiment was carried out with cartilage from 18-day rat embryos. The tibiæ and femora were fragmented and treated in exactly the same way as described previously. As there appeared to be no difference between the survival of 17- and 18-day embryonic cartilage, or between tibiæ and femora, the results were combined and are shown in Table 1.

Table 1. RESULTS OBTAINED AFTER SLOWLY FREEZING EMBRYONIC RAT CARTILAGE FROM 17-DAY AND 18-DAY TIBIÆ AND FEMORA TO – 79° C. THE CARTILAGE WAS PRETREATED WITH TRYPSIN AND SUSPENDED IN 15 PER CENT GLYCEROL-PLASMA

	Killed	Survived	Total
Frozen	4	10	14
Unfrozen	1	9	10
Total	5	19	24

The probability of these results, or more extreme ones, arising from chance, was calculated using Fisher's exact method[10]. The probability was found to be $P = 0{\cdot}566$; thus, although there is a suggestion that the frozen cultures had a greater mortality than the non-frozen cultures, on the data obtained, this is nowhere near significant at the conventional $P = 0{\cdot}05$ level.

Histological examination of the explants which survived the experimental treatments revealed that both the rounded epiphyseal and the hypertrophic diaphyseal cells survived the freezing. The morphology of the cells was normal ; and when the sections were stained with toluidine blue, the characteristic metachromasia of the matrix was seen.

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Temperature Coefficient of Endodiurnal Leaf Movements in *Phaseolus*

THE effect of temperature on the length of cycle of endodiurnal rhythms has been investigated in various processes in plants and animals. It was repeatedly found that within the physiological range the length of cycle is independent of temperature[1-3]. Several other endodiurnal rhythms, however, showed various temperature coefficients between 0·8 and > 3 (refs. 4–6). Went[7,8] reported a temperature coefficient in leaf movements of *Phaseolus* of 1·2 which is in agreement with early observations of Bünning[9]. Went, however, does not state under what conditions his plants were grown before measuring the cycle. As I have pointed out[10], the external conditions under which the seedlings are grown will determine the response. Etiolated plants, grown at 15, 20 and 25° C., showed a constant cycle-length of 28·0–28·3 hr. When the plants were grown in the greenhouse and then transferred to constant conditions, a Q_{10} of 1·2 was observed for the first two cycles ; the Q_{10} changed in the subsequent cycles to 0·9, thus revealing a temperature-compensating mechanism.

Went's statement[7] that my investigations "did not give any temperature effects on the cycle length but showed a constant 24-hr. cycle over a whole range of temperatures" is erroneous. I did not report a 24-hr. cycle in leaf movements. Went implies that a 24-hr. cycle of smoke and fog governed the leaf movements in my investigations. This assumption cannot be maintained since a cycle-length of 28 hr. was observed in etiolated plants. Furthermore, greenhouse-grown plants, measured at 20° C. at constant darkness, showed over five days a constant cycle-length of 26·1–26·7 hr. This finding by Lörcher[11], who used the same facilities as mine, does not reveal effects of external factors operating in regulating the *Phaseolus* clock.

Increased variability in the cycle-length under constant conditions[10] also indicates that the leaf movements were not controlled by external factors.

I showed that greenhouse-grown plants exhibit after transfer to constant conditions in the first two cycles after the transfer a Q_{10} of 1·2 the same coefficient as found by Bünning and Went. Whether or not the length of cycle is altered by temperature, however, is largely dependent on the pretreatment of the seedlings.

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The Northern Rockling in the Clyde Sea Area

ON August 15, 1960, a northern rockling, *Ciliata septentrionalis* (Collett 1875), was caught in the Clyde Sea area. The fish was caught at a depth of 30–40 fathoms in a small mesh cover that had been fitted over the cod end of a trawl used by R.V. *Calanus*. It is 10·6 cm. in length and agrees closely with the descriptions of Smitt[1] and Schnackenback[2].

This is the first record of *Ciliata septentrionalis* from Britain. Elsewhere it has been recorded in Norway between 70° 13′ N. and Bergen[3] and from south Greenland[4]. I have previously found two specimens in a jar in the Sales Department here, but there was no record of when the fish were preserved, who preserved them or where they came from. However, it now seems probable that the fish lives permanently in the Clyde.

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BACTERIOLOGY

Variation of Elasticity along Single Microfibrils of Bacterial Cellulose

A NUMBER of models have been proposed for the internal fine structure of individual cellulose microfibrils which suggest variability both across and along the axis of the microfibril. Frey-Wyssling[1] has suggested that microfibrils are subdivided into four continuous crystalline strands, each about 70 Å. wide, which run parallel to the microfibril axis and are held together by a paracrystalline matrix. In contrast, Preston[2] has concluded that cellulose microfibrils from algæ are composed of a paracrystalline sheath surrounding a highly crystalline single core which is interrupted at random positions by less-crystalline material labile to electron bombardment. The positions occupied by this material may coincide with the sites of trace residues (*Fremdgruppen*) postulated by H. K. Meyer and van der Wyk and by Treiber[3].

In this contribution is presented direct evidence for random variation of elasticity along the length of individual microfibrils of bacterial cellulose.

Cellulose-free, washed cells of *Acetobacter xylinum* were prepared as described previously[4], and 1 ml. of the suspension used to inoculate 35 ml. of sterile medium[5] in Petri dishes, 10-cm. in diameter. Sterile microscope slides were slipped beneath the surface of the medium, and on top of the slides were placed several sterile copper electron microscope grids. Each culture was incubated in covered Petri dishes 4–12 hr. at 30° C. The glass slides carrying the electron microscope grids were gently lifted from the medium (horizontally) and air dried. The dried slides were washed in a slow current of water with a minimum of agitation. The grids with adherent microfibrils were again dried, shadowed with palladium–gold (60 : 40) at an angle of 15° to increase electron scattering and examined.

Examination at low beam intensities showed single straight microfibrils or straight bundles of microfibrils extending over distances of more than 70μ without support. As beam intensity was increased, the single microfibrils or bundles began to coil into rough irregular helices (Fig. 1), presumably because of differential heating of the shadowed and unshadowed halves of the microfibrils. The degree of coiling depended on the maximum intensity to which the microfibrils were exposed and was irreversible.

No appreciable relaxation of the microfibrils to their original straight form was observed on reduction of beam intensity, but an extension or contraction of the coil on variation of beam intensity was often seen. That the coiled microfibrils were under severe longitudinal strain was shown by frequent snapping and sharp recoil of the threads to form tangled clumps (Fig. 1). The twisting of individual microfibrils exhibited their flattish ribbon-like outline clearly (Fig. 2).

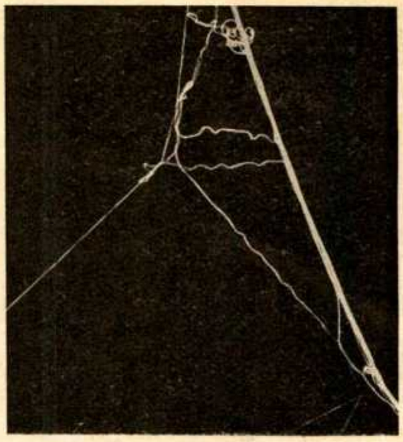

Fig. 1. Helices of bacterial cellulose microfibrils formed by heating shadowed straight microfibrils or bundles in an electron beam. Note tangled clump of microfibrils, formed after breaking in the electron beam

The most interesting property shown by the above helices was the marked variation in coiling of the same microfibril or bundle of microfibrils from point to point along its length (Fig. 3). Not all microfibrils, were coiled, perhaps because of the extreme tension to which some were subjected, but of those which did the pitch of the helix often changed abruptly. Since the total stress was the same at any point of an unsupported segment of a single microfibril, this variation in pitch must reflect a corresponding variation of elasticity at different portions of the thread. Similarly, in a bundle of microfibrils, variation of the pitch of the helix of the bundle must reflect a variation of the composite bundle modulus, which in turn implies variations of the modulus in single microfibrils. Since the angle and degree of metal shadowing, as well as the electron-beam intensity, were uniform along any initially straight microfibril, the above variation in elasticity may be attributed to either a corresponding variation in twisting of the

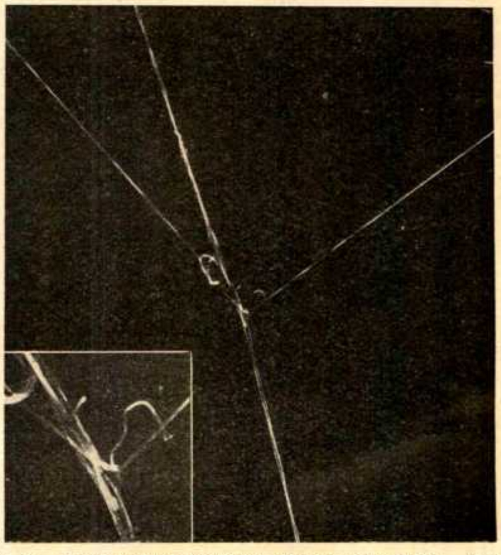

Fig. 2. Single twisted bacterial cellulose microfibrils. Inset: Note end of broken microfibril which may be frayed

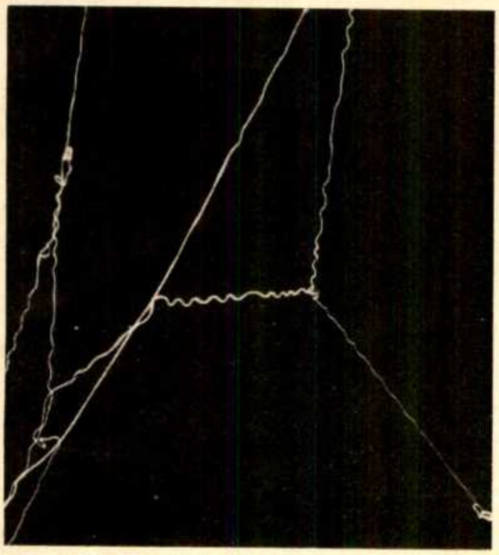

Fig. 3. Helical microfibrils showing the abrupt variation in pitch along their length

original microfibrils or to variations in the fine structure of straight ones. However, if the original microfibrils are irregularly twisted, this also implies a linear variation of internal fine structure. Thus either attribution points to a variation of fine structure along single microfibrils. This variation may be due to differences in crystallinity along the microfibril, differences in the number of β-glucosan chains composing the microfibril at various points, variations in the type of packing of glucose residues within the unit cell or variations in the local content of trace residues along the microfibril.

The random variation of tensile strength along microfibrils of bacterial cellulose suggests that their fine structure is closer to the model proposed by Preston for *Valonia* cellulose than that suggested by Frey-Wyssling, since variations along four crystalline continuous sub-filaments would tend to cancel out. Also, if sub-filaments existed in single microfibrils of bacterial cellulose, fraying on breakage should be evident; but only one tentative instance out of several hundred was noted (Fig. 2). This single observation may also be attributed to irregular fracture along a solid core and is therefore not conclusive.

Since bacterial cellulose microfibrils grow at the tips[4,6], the observed variation in tensile properties implies corresponding deviations in the detailed mechanism of deposition and crystallization of individual glucose residues during fibrogenesis. Such deviations, however, must be only digressions from a stable process since clearly they are corrected quickly. The inference of such temporary deviations is also supported by Ränby's recent observation that bacterial cellulose microfibrils which have never been dried have approximately 25 per cent more accessibility to tritium atoms than the same material after drying[7].

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GENETICS

Genetic Recombination in *Vibrio cholerae*

THE occurrence of genetic recombination in *V. cholerae*, revealed by crosses between double auxotrophic mutants of strain 162, was mediated by a fertility factor—designated the *P* factor—which could be infectively transmitted from strain to strain by conjugation[1]. P^+ strains (possessing the fertility factor) gave rise to recombinants when crossed with P^- strains (devoid of the fertility factor).

The relative roles of P^+ and P^- strains in such crosses have now been elucidated. When P^+ strains, suspended in minimal medium, were exposed to ultra-violet light to reduce their viable count by a factor of 1/1,000 to 1/10,000 and then crossed with normal P^- strains there was a considerable stimulation of recombination as judged by the number of colonies which appeared on selective media. If, on the other hand, P^- strains were likewise exposed to ultra-violet light to effect a similar reduction in viable count and then crossed with normal P^+ culture, recombination was almost completely abolished. This differential effect of ultra-violet light has been confirmed in four different crosses between P^+ and P^- mutant stocks. Table 1 furnishes details of one such cross. P^- strains grown in broth culture supernatants of

Table 1. EFFECT OF ULTRA-VIOLET IRRADIATION OF PARENT STRAINS ON GENETIC RECOMBINATION IN *Vibrio cholerae* CROSS $V44P^+$(*pur*, *arg*, *str-r*, 200-*r*) × $V39P^-$ (*ilva*, *str-s*, 200-*s*)

Cross	No. of viable cells of each parent in the cross	No. of colonies (in duplicate plates)			
		purine minimal agar		arginine minimal agar	
$V44P^+ \times V39P^-$	$1\cdot7 \times 10^8 + 2\cdot5 \times 10^8$ $6\cdot0 \times 10^7 + 8\cdot0 \times 10^7$	193 74	191 57	25 10	25 11
$V44P^+$ (U.V.) × $V39P^-$	$1\cdot0 \times 10^4 + 2\cdot5 \times 10^8$ $3\cdot0 \times 10^3 + 8\cdot0 \times 10^7$	*c.* 1,000 150	*c.* 1,000 154	232 56	253 54
$V44P^+$ × $V39P^-$ (U.V.)	$1\cdot7 \times 10^8 + 2\cdot3 \times 10^4$ $6\cdot0 \times 10^7 + 8\cdot0 \times 10^3$	nil nil	nil nil	nil nil	nil nil
$V44P^+$(U.V.) × $V39P^-$ (U.V.)	$1\cdot0 \times 10^4 + 2\cdot3 \times 10^4$ $3\cdot0 \times 10^3 + 8\cdot0 \times 10^3$	3 nil	4 nil	nil nil	nil nil

U.V., irradiated with ultra-violet light; 200-*s*, sensitivity to phage 200; 200-*r*, resistance to phage 200; *pur*, *arg*, requirement for purine and arginine; *ilva*, requirement for valine and *iso*leucine; *str-s*, sensitivity to streptomycin; *str-r*, resistance to streptomycin.

irradiated P^+ cultures did not give rise to recombinants.

The differential lethal effect of ultra-violet light on P^+ and P^- strains shows that recombinants arise from the P^- parent, which probably functions as a gene-acceptor. P^+ strain appears to be a gene-donor, the viability of which is not essential for the expression of the recombinants.

A similar situation is now well recognized in *Escherichia coli*[2,3] in which it was first demonstrated by the differential effect of streptomycin[4] on F^+ and F^- strains. The streptomycin method was not suitable for *V. cholerae*, as it resulted in a marked reduction in recombination-rate. Nevertheless, a significant number of recombinants could be obtained when P^+ strains were treated with streptomycin and crossed with P^- cultures and none at all if P^- strains were treated with streptomycin and crossed with P^+ cultures. Full details of this work will be published elsewhere.

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Histocompatible Colour Mutant from *CBA/Lab* Strain of Mice

The progeny from a breeding pair of *CBA/Lab* mice, a strain which is propagated by brother × sister mating, were found to have grey agouti coats in contrast to the usual wild-type agouti of the strain. These '*CBA* grey' mice when mated together produced similar 'grey' mice among their progeny (that is, the 'grey' character behaved as if it were determined by a recessive gene), and it was thought to be of interest to test the original strain and its mutant for histocompatibility.

The results are shown in the photographs of skin grafts from normal *CBA* and mutant animals on a *CBA* mouse (Fig. 1) and on a mutant *CBA* mouse (Fig. 2) indicate histocompatibility between the two lines. A similar compatibility to skin grafting between a *CBA* mutant *p* (pink eye) and its host was found by Fraser and Clayton[1]. In a later communication given in the Institute of Animal Genetics Research Report 1955–1957, Mrs. Clayton refers to antigenic differences between these two types.

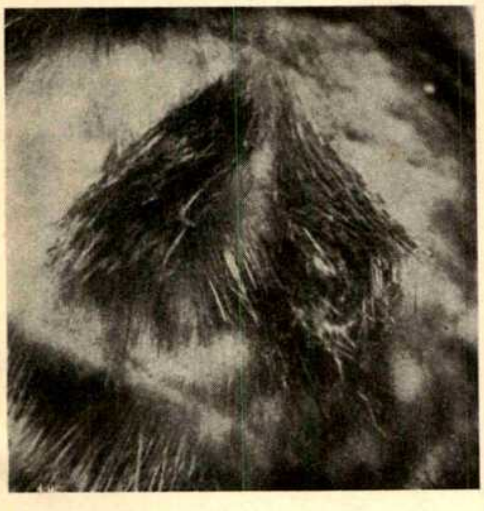

Fig. 1

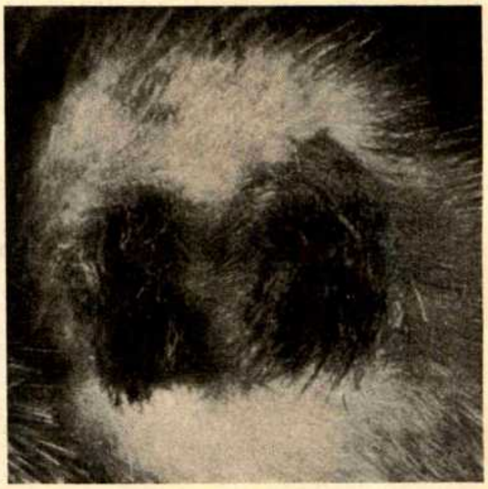

Fig. 2

Preliminary tests by Dr. Mary Lyon of the Radiobiological Research Unit, Harwell, to determine the gene that our mutant carries have shown that grey is not the same as or allelic with genes *a*, *b*, c^{ch}, *d*, *pa* or *p* (pink eye). This does not necessarily mean that it is a new mutant, and further tests are progressing

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PSYCHOLOGY

Theory of the Visual Threshold

Gregory[1] and Cane and Gregory[2] have put forward a theoretical equation for the brightness increment threshold considered as a statistical discrimination. For a given frequency of detection they propose that:

$$\frac{[(r + \Delta r) - r] - C}{\sqrt{\left(\frac{1}{A_1} + \frac{1}{A_2}\right) V}} = \text{const.} \qquad (1)$$

where $(r + \Delta r)$, r, are the mean neural pulse-rates associated with the intensities $(I + \Delta I)$, I, and V is the variance of these rates. C is a constant necessary to account for the occurrence of fewer than 50 per cent false positives. Its implication, as stated by Gregory, is that "some fixed difference between impulse rates is required before discrimination is established, this difference being independent of the intensity I".

This equation can be regarded as an adaptation of Thurstone's[3] law of comparative judgment, case 3, for answers of 'yes' or 'no' in place of comparisons. The term:

$$\sqrt{\left(\frac{1}{A_1} + \frac{1}{A_2}\right) V}$$

can be regarded as the standard deviation of the distribution of $[(r + \Delta r) - r]$, assuming the two terms to be uncorrelated, and will be further referred to as σ_r. The constant will be called b.

Marriott[4] has shown that this equation leads to rather implausible consequences: (1) Although when the threshold is defined by a criterion such as 80 per cent of positive responses, ΔI will decrease as the area increases (causing σ_r to decrease), as Gregory[1] found, this will not be true when the criterion is 50 per cent positive responses, in which case threshold will be independent of changes in area. (2) With a criterion such as 20 per cent, ΔI decreases as the area decreases (σ_r increases). It might be added (3) that though the threshold will stay constant despite changes in area with a 50 per cent criterion, the false positive rate will decrease as the area increases. Consequently, Marriott rejects the approximations to Ricco's and Piper's laws for areal summation at the absolute threshold which Cane and Gregory derive from their assumptions.

The modification of the equation in a way suggested by the theory of signal detectability appears capable of avoiding these difficulties. In place of Gregory's assumption that a fixed difference between impulse-rates (or whatever central effects of the stimuli determine the responses) is required by the brain to establish discrimination, let us suppose that the discrimination mechanism operates to give maximum detection consistent with a limiting rate of false positive responses. In this case the value of C will not be constant, but must be defined as a function of σ_r. Let us therefore replace it by $m\sigma_r$, where the value of m determines the proportion of false positives. Thus, when $m = 2$, the false positive rate would be approximately equal to 2·5 per cent. The equation for the threshold can now be expressed as:

$$[(r + \Delta r) - r] = (m + b)\sigma_r \qquad (2)$$

Assuming that the distribution of $[(r + \Delta r) - r]$ is approximately normal, b is the normal deviate corresponding to the frequency of response required to define the threshold. When this is 50 per cent, $b = 0$ and the right-hand terms reduce to $m\sigma_r$. Thus the threshold is still directly proportional to σ_r, so that it will vary with area just as when it is defined by an 80 per cent requirement. This meets Marriott's first point. When the requirement is 20 per cent, the equation becomes $(r + \Delta r) - r = (m - b)\sigma_r$, where b is the normal deviate corresponding to 80 per cent negative responses. In this case too the threshold will decrease as σ_r decreases, that is, as the area increases, thus meeting the second objection. Moreover, so long as the response-rate required to define the threshold exceeds the false positive rate, $(m - b)$ will be positive.

Even though the threshold equation in the form above meets Marriott's objections, there is still reason to doubt the derivation of Ricco's and Piper's laws, based on the assumption that r is proportional to $\log(I + k)$, which is offered by Cane and Gregory, where k is a constant assumed by Gregory to represent the internal noise of the system. A very similar result is given by equation (2) to that obtained by Cane and Gregory with equation (1). At the absolute threshold, where $I = 0$, taking exponents in equation (2), and ignoring A_2, which is the total visual field, we get:

$$1 + \frac{\Delta I}{k} = e^{(m+b)V^{1/2}A^{-1/2}} \qquad (3)$$

Defining the unit of I so that $k = 1$, and putting $(m + b)V^{\frac{1}{2}} = d$, we get for the absolute threshold:

$$\Delta I = d/A^{1/2} + d^2/2A + d^3/6A^{3/2} \ldots \qquad (4)$$

Cane and Gregory obtain Ricco's law by multiplying throughout by A, and Piper's law by multiplying by $A^{1/2}$. In each case a constant appears among the terms on the right. However, it has been pointed out to me by Dr. Marriott (private communication) that in the first case it is the second term (as given in equation (4)) which provides the constant and in the second case the first term. This causes the following difficulty: the values of the terms on the right depend on the values of both d and A. While d and A can have values such that the first term is considerably larger than any other term, and decrease in the value of A will increase the size of the second term relative to the first term, it will cause the values of other terms to increase as well, and at no time will the second term be more than a fraction of the total value of ΔI (for example, if $d = 1$, $A = 10$, the first terms are 0·3, 0·05, 0·005. . . . If A is made 0·1, the terms are 3·2, 5·0, 5·3 . . .). Thus the unmodified assumption of a simple logarithmic transformation of the stimulus, so far from leading to Ricco's law, appears to exclude it.

I wish to thank Prof. R. C. Oldfield for research facilities, and Dr. C. I. Howarth and Dr. F. H. C. Marriott for helpful discussions.

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SOCIOLOGY

Conflicting Hypotheses on the Growth of World Population

Over the past twenty thousand years, the logarithm of human population of small areas such as England and Wales—when plotted against time—shows two or more special upsurges, or kinks; for example, due to the introduction of agriculture, or since A.D. 1500. One may recognize three main demographic stages[1]. With the same idea, Prof. E. S. Deevey of Yale proposes a striking plot of total world population, featuring two such kinks in a very pronounced manner[2]. But the "overall geographical speed of cultural diffusion", in early times, was slow: often between 0·5 and 5 km. per annum[3]. Consequently different small areas had their main kinks at widely different times; and they will practically disappear on summation. Hence, with due allowance for the different scales used, the relatively smooth curve for world population[4] seems likely to be much nearer the truth than Deevey's of the following September.

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FORTHCOMING EVENTS

*(Meeting marked with an asterisk * is open to the public)*

Monday, March 27

British Institution of Radio Engineers, Medical Electronics Group (at the Postgraduate Medical School, University of London, Hammersmith, London, W.12), at 3 p.m.—Symposium on "Electronic Instrumentation for Cardiac Surgery".

Royal Geographical Society (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. G. de Q. Robin : "The Antarctic Ice Sheet".

Institution of Mechanical Engineers, Internal Combustion Engines Group (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Is Fuel-Injection Equipment Standardization Limiting the Development of Small Diesel Engines?".

Operational Research Society (in the Lecture Theatre of the Royal Society of Arts, John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Mr. D. T. Steer : "Feasibility and Financial Studies of a Port Installation".

Monday, March 27—Wednesday, March 29

Biochemical Society (in London)—Fiftieth Anniversary Meeting.

Monday, March 27—Tuesday, March 28

(In the Senate House, University of London, W.C.1)—Symposium on "Structure and Synthesis of Macromolecules".

Tuesday, March 28

(At the Royal Institution, Albemarle Street, London, W.1)—Sir Hans Krebs : "The Physiological Role of Ketone Bodies" (Third Hopkins Memorial Lecture).

Wednesday, March 29

(At University College, Gower Street, London, W.C.1)—Original Communications and Annual General Meeting.

Tuesday, March 28

Society of Instrument Technology, Control Section (at 26 Portland Place, London, W.1), at 5 p.m.—Dr. P. E. W. Grensted and Mr. O. L. R. Jacobs : "Automatic Optimization". 7 p.m.—Mr. P. F. Sutherby : "The Development of an Optimizing Controller".

Royal Society of Medicine, Medicine Section (at 1 Wimpole Street, London, W.1), at 8 p.m.—Meeting on "Isotopes in Medicine" Speakers : Prof. G. M. Wilson, Prof. D. W. Smithers and Dr. E. Williams.

Tuesday, March 28—Thursday, March 30

Royal Institution of Naval Architects (in the Weir Lecture Hall, 10 Upper Belgrave Street, London, S.W.1), at 10.15 a.m. daily —Spring Meeting.

Wednesday, March 29

British Institution of Radio Engineers (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 3 p.m.—Symposium on "Electronic Instrumentation for Nuclear Power Stations".

Institution of Mechanical Engineers (in conjunction with the Steam Plant Group, at 1 Birdcage Walk, Westminster, London, S.W.1), at 3.30 p.m.—Mr. R. W. Haywood, Mr. C. A. Knights, Mr. C. E. Middleton and Mr. J. R. S. Thom: "An Experimental Study of the Flow Conditions and Pressure Drop of Steam-Water Mixtures at High Pressures in Heated and Unheated Tubes".

Society for Analytical Chemistry (at the Chelsea College of Technology, Manresa Road, Chelsea, London, S.W.3), at 6 p.m.—Demonstration of Laboratory-Made Equipment".

Royal Aeronautical Society, Agricultural Aviation Group (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. R. P. Fraser : "Atomization".

Royal Institute of Chemistry (at Hendon Technical College, The Burroughs, London, N.W.4), at 7.30 p.m.—Dr. K. G. A. Pankhurst: "The Story of Soap and the Growth of Modern Synthetic Detergents".*

APPOINTMENTS VACANT

Applications are invited for the following appointments on or before the dates mentioned :

Senior Research Assistant (postdoctoral standard, qualified in inorganic chemistry), to take part in an investigation of the crystal chemistry of oxides and hydroxides of the transition metals—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (April 5).

Research Fellow (with a first degree in biochemistry, chemistry or botany, and research experience in biochemistry and particularly in the use of radioactive tracers) in Plant Biochemistry in the Department of Botany, to participate in research on the mechanism of photosynthesis—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (April 7).

Lecturer or Assistant Lecturer in Zoology—The Secretary, The University, Exeter (April 10).

Zoology Assistant (Grade B) (with good academic qualifications) in the Department of Botany and Zoology—The Clerk to the Governors, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 10).

Assistant in Physical or Inorganic Chemistry—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (April 15).

Chair of Agriculture; Chair of Physics; Chair of Education; and Chair of Geography—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas (Dept. T.1), 29 Woburn Square, London, W.C.1 (April 15).

Lecturer in Microbiology; and a Lecturer or Assistant Lecturer in Pharmacology at the University College of the West Indies—The Secretary, Senate Committee on Colleges Overseas in Special Relation, University of London, Senate House, London, W.C.1 (April 17).

Chair of Zoology tenable in the Durham Division of the University —The Registrar, University Office, 40 North Bailey, Durham (April 22).

Organic Chemist (graduate, or holder of a technical college diploma majoring in organic chemistry, and preferably with experience of distillation and chromatographic techniques) in the Division of Coal Research, C.S.I.R.O., Sydney, Australia, to assist on investigations of the chemical constitution of coal tar and pitch and in the development of new industrial uses for these materials—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 480/359 (April 22).

Imperial Chemical Industries Research Fellows in Biochemistry, Chemistry, Chemotherapy, Engineering, Metallurgy, Pharmacology or Physics—The Academic Registrar, University of London, Senate House, London, W.C.1 (April 25).

Lecturer in Mathematics at the University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 25).

Senior Lecturer in Physics; a Senior Lecturer (graduate with Corporate Membership of the Institution of Electrical Engineers) in Electrical Engineering; a Lecturer in Botany; a Lecturer or Assistant Lecturer (preferably with qualifications in physical and/or inorganic chemistry) in Chemistry; and a Lecturer (preferably with qualifications and/or experience in palæontology, stratigraphy or structural physical geology) in Geology, at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 25).

Assistant Lecturer (honours graduate) in Physiology; and an Assistant Lecturer (honours graduate) in Biochemistry—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (April 28).

Lecturer in Philosophy at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, April 28).

Senior Lecturer/Lecturer (preferably with previous research experience) in the Department of Statistics of the School of Mathematics, University of New South Wales, Kensington—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2 (Australia and London, April 30).

Burn Research Fellow (graduate, not less than thirty years old, with two or three years research experience) in Pharmacology at Oxford—Secretary to the Trustees, Department of Pharmacology, University of Oxford (May 15).

Lecturer or Senior Lecturer in Mathematics at the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 15).

Chair of Agriculture in the University of Tasmania, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

McCaughey Chair of Geography in the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 31).

Assistant Lecturer (preferably with research experience in some field of experimental zoology) in the Department of Biology as Applied to Medicine—The Secretary, Department of Biology as Applied to Medicine, Middlesex Hospital Medical School, London, W.1 (July 1).

Lecturer and an Assistant Lecturer in the Department of Anatomy—The Secretary, University College London, Gower Street, London, W.C.1.

Master to teach Mathematics to G.C.E. "O" level—The Headmaster, Queen Elizabeth's Grammar School, Blackburn.

Reader, and a Lecturer, in Low Temperature Physics—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

Research Assistants (with a degree in chemistry or chemical engineering, or with equivalent qualifications such as Grad.R.I.C.) in the Department of Chemistry, for experimental work on the thermodynamic properties of gases and of liquid mixtures—The Bursar, The University, Reading.

Research Chemist (with experience of research work in physical chemistry or chemical engineering, and preferably with a higher degree in one of these subjects) in the Department of Chemistry to continue a study of the thermodynamics of imperfect gases, under the items of a contract between the University and the D.S.I.R.—The Registrar (Room 22 O.R.B.), The University, Reading.

Research Consultant (with sound British academic qualifications, and specialized in physical or inorganic chemistry) in the Centre of Chemistry at the University of Chile, Santiago, to conduct and advise on research, advise on the organization of the Centre, and to conduct courses for the research staff—The Director, Recruitment Department, The British Council, 65 Davies Street, London, W.1, quoting Ref. N.4.T(61).

Whole-time Biochemist (non-resident), for hospital biochemical work in the Mansfield Area Pathology Service—The Hospital Secretary, King's Mill Hospital, Sutton-in-Ashfield, Notts.

AC 80

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Organometallic Compounds, No. 1. A new abstract journal. Published monthly. Subscription rates: £6 per year U.K. and Overseas. Airmail postage Overseas £9. United States and Canada 18 dollars seamail, 27 dollars airmail. Pp. i+25. (London: Translation and Technical Information Services, 32 Manaton Road, S.E.15, 1961.) [2012

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Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 10: Apple Capsids. Pp. 4. Leaflet No. 89: Some Pests and Diseases of Vegetable Crops. Pp. 13. (Belfast: Ministry of Agriculture, 1960.) [2012

Agricultural Research Institute of Northern Ireland. Thirty-third Annual Report, 1959-1960. Pp. 47. (Hillsborough, Co. Down: Agricultural Research Institute of Northern Ireland, 1960.) [2012

The Institute of Physics and the Physical Society. Handbook of Scientific Instruments and Apparatus, 1961 Exhibition. Pp. xvi+335+A75. (London: Institute of Physics and the Physical Society, 1960.) [2012

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Tweed River Purification Board. Annual Report for the year ended 15th May, 1960. Pp. 39. (Newton St. Boswells: Tweed River Purification Board, 1960.) [2012

Privy Council. Medical Research Council Special Report Series, No. 297: The Composition of Foods. By Dr. R. A. McCance and Dr. E. N. Widdowson. (Third revised edition of Special Report No. 235). Pp. viii+252. (London: H.M. Stationery Office, 1960.) 30*s.* net. [2012

University Grants Committee. Methods used by Universities of Contracting and of Recording and Controlling Expenditure. Pp. 28. (Cmnd. 1235). (London: H.M. Stationery Office, 1960.) 1*s.* 6*d.* net. [2212

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Medical Research Council. The Hazards to Man of Nuclear and Allied Radiations—a Second Report to the Medical Research Council. Pp. vii+154. (Cmnd. 1225). (London: H.M. Stationery Office, 1960.) 7*s.* net. [3012

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Other Countries

National Science Foundation. Scientific and Technical Personnel in American Industry: Report on a 1959 Survey. Pp. vi+66. (Washington, D.C.: Government Printing Office, 1960.) 45 cents. [2012

Mitteilungen aus der Biologischen Bundesanstalt für Land—und Forstwirtschaft, Berlin-Dahlem. Heft 100 (Oktober 1960): Das Trichogramma-Problem. Pp. 50 (4 tafel). (Berlin-Dahlem: Biologische Bundesanstalt für Land—und Forstwirtschaft, 1960.) 9.60 D.M. [2012

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United States Department of the Interior: Geological Survey. Bulletin 1072-N: Stratigraphy of the Little Rocky Mountains and Encircling Foothills, Montana. By Maxwell M. Knechtel. Pp. iv+723-752+plates 52 and 53. Bulletin 1072-P: Coal Resources of Arkansas, 1954. By Boyd R. Haley. Pp. iv+795-831+plates 58-64. Bulletin 1087-F: Geology and Ore Deposits of the Kern River Uranium Area, California. By E. M. MacKevett, Jr. Pp. iv+169-222+plates 19-25. Bulletin 1087-H: Geology of the Clay Hills Area, San Juan County, Utah. By Thomas E. Mullens. Pp. iv+259-336+plate 27. 75 cents. Bulletin 1096-A: Geology of the Grosvenor Quadrangle, Brown and Coleman Counties, Texas. By Robert T. Terriere. Pp. iv+35+plates 1-3. Bulletin 1072-J: Reconnaissance Geology of the Birney-Broadus Coal Field, Rosebud and Powder River Counties, Montana. By W. C. Warren. Pp. iv+561-585+plates 19-26. (Washington, D.C.: Government Printing Office, 1959 and 1960.) [2012.

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